REVIEW

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Challenges in evidencing the earliest traces of life

Emmanuelle J. Javaux[1]*

Earth has been habitable for 4.3 billion years, and the earliest rock record indicates the presence of a microbial biosphere by at least 3.4 billion years ago—and disputably earlier. Possible traces of life can be morphological or chemical but abiotic processes that mimic or alter them, or subsequent contamination, may challenge their interpretation. Advances in micro- and nanoscale analyses, as well as experimental approaches, are improving the characterization of these biosignatures and constraining abiotic processes, when combined with the geological context. Reassessing the evidence of early life is challenging, but essential and timely in the quest to understand the origin and evolution of life, both on Earth and beyond.

Analyses of highly resistant microscopic minerals (zircons) preserved in younger host rocks indicate that Earth was already habitable—that is, had liquid water at the surface or in near-surface environments, as well as some crust—by 4.3 billion years ago (Ga)[1]. Life could have originated at this time but it is only possible for us to test scenarios for the origin of life experimentally, because the transition from molecules to the first cells is unlikely to be preserved. Moreover, the preserved rock record starts only around 4 Ga. These old rocks, which could potentially preserve early traces of life, have been much altered by geological processes, which makes the interpretation of the environmental conditions of their formation and initial composition challenging. Nevertheless, the ancient record provides evidence for the presence of a crust, rock erosion and recycling, and liquid water[1,2], which suggests that life could have accessed nutrients, energy sources and habitats early on.

The incompleteness of the rock and fossil records is the first challenge that palaeobiologists and geologists face when searching for early traces of life (Fig. 1). Rocks are transformed, eroded and recycled through time, and fossilization processes are complex and rare. Consequently, the overall proportion of rocks and fossils that are preserved from the early Earth is small, and offers only a glimpse of the histories of our planet and the evolution of life. The possible traces of life that have been reported in early-Earth sediments and rocks are morphological or chemical (Fig. 2). Chemical traces include modified molecules that are originally produced only by biological activity (such as complex lipids and pigments), as well as the isotopic composition of carbon, sulfur, nitrogen and iron, which is indicative of the biological cycling of these elements. Morphological traces comprise microfossils and biosedimentary structures such as stromatolites (laminated rocks produced by microbial precipitation and/or trapping of minerals) in carbonate rocks and other microbially induced sedimentary structures in siliciclastic rocks. Traces of biological activity, such as microbial borings, burrows and tracks (ichnofossils), can indirectly indicate the presence of life but their biological origin is difficult to prove, especially in the Archean eon (the time period from 4 to 2.5 Ga). Many similar traces can also be formed and/or altered by abiotic processes or later contamination, which has left controversies surrounding the earliest record of life on Earth[3–17].

Challenges

The geological context

The first step in recognizing biosignatures is determining the geological context[18] and the environmental conditions of preservation. The samples that host the potential traces of life should come from rocks of known provenance, of established age and demonstrated geographical extent. Moreover, the possible traces should occur in a geological context that is plausible for life; these contexts are mostly in sedimentary environments[9,19–22], although some putative microbial borings have been reported in marine volcanic glass[23] (but were later reassessed as abiotic[24,25]). Fossilization is a complex process that involves microbial, physical and chemical degradation that leads to the decay, preservation and alteration of original biological information and properties, and varies according to the environmental conditions of preservation and the geological processes that affect the rocks. Low-temperature biological, chemical and physical processes of compaction and cementation (diagenesis) transform soft sediments into coherent rocks. High pressure and temperature processes owing to increasing burial depth or deformation (metamorphism), hot fluid circulation (hydrothermalism and metasomatism), and folding and fracturing (tectonics) may modify, alter or erase the original composition and fabric of the rocks, and any biosignatures that they may preserve. The occurrence and extent of these processes must be characterized before any attempt at meaningful interpretation.

Observations at different scales—from sedimentary basins (tens to hundreds of kilometres) through outcrops (kilometres to metres), bedrocks (metres) and laminations (centimetres to millimetres) to microscopic examinations of rock samples (micrometres to nanometres)—and using diverse geochemical proxies help to constrain the geological context and the particular environmental conditions of preservation. These analyses provide key information on the age, composition and origin of rocks and fossilized carbonaceous material, on fossilization processes in diverse physicochemical conditions, and on possible alterations. These analyses are also crucial for evaluating the plausibility of abiotic processes, and for assessing the biological or abiotic origin of putative biosignatures.

Endogenicity and syngenicity of life traces

Any purported ancient microfossils or other traces of life must pass two tests before they can be considered as possible evidence of early life. Rocks can be dated directly, but fossils or other life traces cannot; their relationship to the rock must, therefore, first be established. Microorganisms or organic material can enter existing rocks through borings or fluids in veins and pores[9]. Careful microscopic examination of thin sections (petrography) cut through the rock sample can exclude

[1]Early Life Traces & Evolution–Astrobiology, UR Astrobiology, Department of Geology, University of Liège, Liège, Belgium. e-mail: ej.javaux@uliege.be

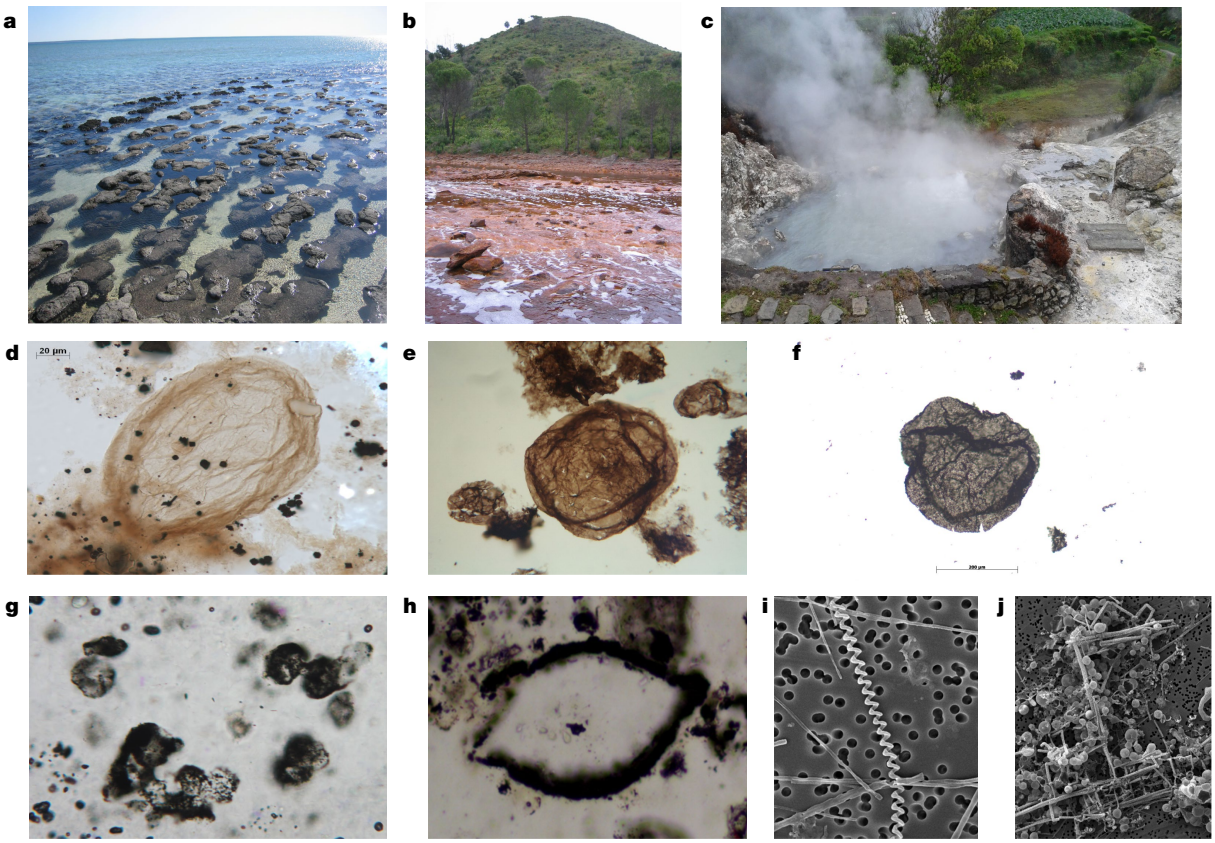

Fig. 1 | Challenges in studying traces of early life. These challenges include the incompleteness of the rock and life records, their preservation and alteration, contamination, interpretation, and abiotic processes altering or mimicking biosignatures. **a**–**c**, Examples of modern environmental conditions that affect the preservation of biosignatures. **a**, Carbonate shallow-water marine environment with stromatolites (Shark Bay, Australia). **b**, Acidic iron-rich river (Rio Tinto, Spain). **c**, Hot springs (Acores, Portugal). **d**–**f**, Carbonization effect of increasing metamorphism on organic-walled microfossils (50–200 μm in diameter) preserved in non-metamorphosed shales from the 1.1-Ga El Mreiti Group (Mauritania) (**d**), the 1.5-Ga Roper Group (Australia) (**e**) and the 3.2-Ga Moodies Group (South Africa) (**f**). **g**–**j**, Pseudofossils formed by natural and artificial abiotic processes. **g**, Ten-micrometre-diameter pseudofossils formed by the migration of biological or abiotic organic matter around minerals in hydrothermal chert from the 755–735-million-year-old Callison Lake Formation (Canada). **h**, One-hundred-and-fifty-micrometre-long abiotic biomorphs formed by volcanic glass in the Dresser Formation (Australia). **i**, **j**, Nanometre-to-micrometre-scale spherical-to-tubular (sometimes spiralling) biomorphs from abiotic autoassembly of minerals and organic molecules in laboratory experiments. Images from E.J.J. (**a**–**f**) or courtesy of A. H. Knoll (**g**), D. Wacey (**h**) or J. Cosmidis (**i**, **j**).

fluid migration, and can reveal whether the microstructure or carbonaceous material is indeed endogenous (occurs within the rock) as well as whether it is syngenetic (contemporaneous) with the rock[26]. Raman spectroscopy provides evidence of whether the microstructure, putative microfossil or other material is carbonaceous in nature, and of the temperature to which it has been subjected during metamorphism[10,17,21,27]. This temperature should be consistent with the thermal maturity of the associated carbonaceous material co-occurring in the same rock sample, and with the degree of metamorphism of the host rock, which is itself established by the mineralogy, deformation and regional context of this rock. Raman spectroscopy is thus necessary, but not sufficient, to prove biogenicity[28]. An organic microstructure incorporated in fine-grained sediments (such as mudstone or shale) at the time of sedimentation should be flattened parallel to the bedding during sediment compaction, which provides another criterion for syngenicity[21]. If these tests of endogenicity and syngenicity are successful, the challenge then lies in demonstrating the biogenicity of the structure.

Abiotic processes that mimic life

Another difficulty in identifying unambiguous traces of early life is the occurrence of abiotic processes that may mimic life morphologies and chemistries[12]. At least since Wöhler's artificial synthesis of urea in 1828, scientists have known that 'organic' is not a synonym for 'biogenic'. Organic molecules (those that contain carbon–hydrogen bonds) can form in the laboratory or in nature, both on Earth and beyond Earth in the interstellar medium, meteorites and comets. Nonetheless, confusion remains in the scientific and public literature, which can lead to over-interpretation. Organic or carbonaceous material can occur as molecules that are insoluble (kerogen) or soluble (bitumen) in organic solvents[3]. Diverse abiotic processes are known to form gaseous and liquid carbonaceous materials—some of which have carbon and nitrogen isotopic compositions that are similar to those of signatures of life—at low and high temperatures, during metamorphism[7,29], in hydrothermal[6,30] or volcanic[31] environments, and in meteorites[32,33]. Recently, low temperature alteration of the oceanic crust was shown to produce a range of types of condensed carbonaceous particles[34]. The possibility that this abiotic carbonaceous material could mature through burial, diagenesis and metamorphism into abiotic kerogen-like material—although plausible—remains to be tested experimentally[35,36]. Nevertheless, abiotic kerogen, which is associated with a large fractionation of carbon isotopes, is found in carbonaceous meteorites (although its formation is not well-understood)[37]. Kerogen is preserved in many organic-rich Precambrian rocks as particles; it may also form the organic walls of microfossils, in which biopolymers have been transformed by diverse degradation and condensation reactions[3]. Other abiotic processes can also fractionate stable isotopes of carbon, sulfur, nitrogen and iron; these include UV dissociation (for sulfur), electric discharge in the laboratory or lightning in nature (for nitrogen) and mineral precipitation[6,38–40]. The isotopic compositions can then be altered by geological

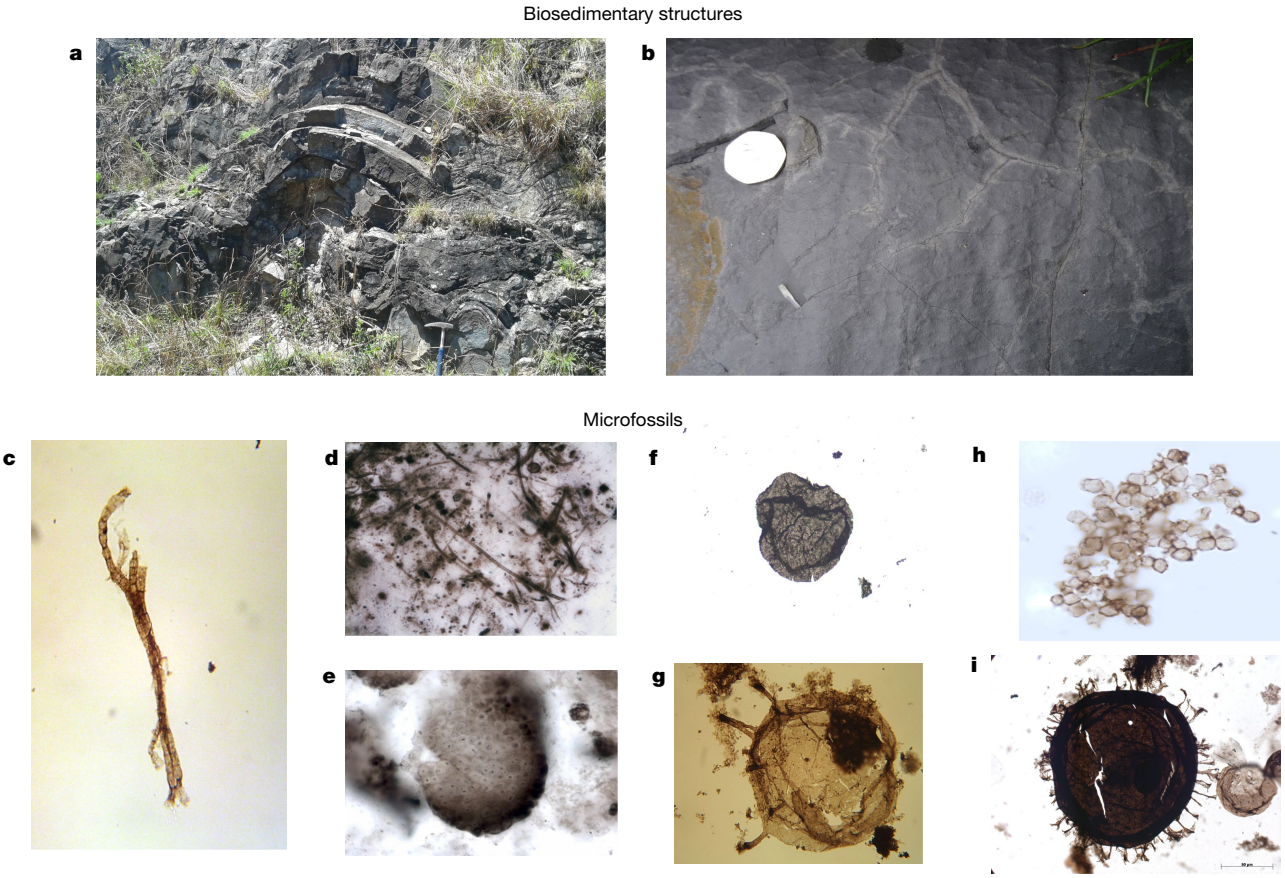

Fig. 2 | Traces of life. Possible traces of life reported in early Earth sediments and rocks can be morphological or chemical. **a**, **b**, Morphological traces include biosedimentary structures produced by microbial mats, such as stromatolites (2.5 Ga, South Africa; hammer for scale) in carbonate rocks (**a**) and microbially induced sedimentary structures (1-Ga Torridonian Supergroup, Scotland) in siliciclastic rocks (**b**). **c**–**i**, Microfossils comprise another major type of morphological trace; **c**, **f**–**i**, preserved as carbonaceous compressions in shales; **d**, **e**, preserved three-dimensionally in chert. **c**, Filamentous bacteria, 100-μm long (1.45-Ga Roper Group, Australia). **d**, Filamentous and coccoidal microfossils (1.9-Ga Gunflint Formation, Canada). **e**, *Eoentophysallis*, colonial cyanobacteria (1.9-Ga Belcher Group, Canada). **f**, Spheroidal microfossil, 220 μm in diameter (3.2-Ga Moodies Group, South Africa). **g**, Protist, 90 μm in diameter (1.45-Ga Roper Group, Australia). **h**, Colony of 2-μm-diameter coccoidal microfossils (1 Ga, El Mreiti Group, Mauritania). **i**, Protist, 100 μm in diameter (1.65-Ga Ruyang Group, China). Images by E.J.J. (**a**–**c**, **f**–**i**) or courtesy of K. Lepot (**d**) or C. Demoulin (**e**). Chemical traces include isotopic composition of carbon, sulfur, nitrogen, iron and molecules such as complex pigments and lipids.

processes such as metamorphism, metasomatism and hydrothermalism, during or after deposition[6,38].

Another complication is the fact that structures that resemble microfossils or stromatolites can also form abiotically. Folded layered rocks or mineral precipitates can be confused with biogenic stromatolites[4,8,11,19,41–45] and can be produced in laboratory abiotic experiments[46] or by numerical modelling[47]. Both abiotic and biological organic matter can migrate around quartz minerals in chert (silica-rich rock)[26,48] or volcanic glasses[49] to create abiotic pseudofossils, some of which are similar in morphology to putative silicified spheroidal or filamentous microfossils[5,50–53] (Fig. 1). Microscopic observations can sometimes help to detect fluid migration[26] and to discriminate between biotic and abiotic microstructures, when the distribution of organic matter clearly follows the angular morphology of a mineral[15]. Mixtures of elemental sulfur with carbon, and carbonate, phosphate or silicate minerals can auto-assemble to form an amazing diversity of objects—sometimes very complex—that mimic life morphologies, in the presence or absence of abiotic or biotic organic molecules (Fig. 1i, j). These 'biomorphs' may form naturally (under Earth conditions[54,55] and in the Martian meteorite ALH84001[56]) as well as in laboratory experiments[57–59]. In some cases, these biomorphs differ from true microfossils in their size distribution and the chemical conditions that are required for their formation[59]. Whether they can have coupled isotopic compositions of carbon, nitrogen, sulfur and iron that are consistent with biological populations, as well as an ultrastructure that is comparable to fossil cell walls, remains to be tested. It is possible that these mineral biomorphs—if associated with organics—could be fossilized as three-dimensional pseudofossils in chert[49,60] but not as organic compressions[21] in fine-grained sediments, as are true organic-walled microfossils (Figs. 1d–f, 2c, f, h) or eukaryotic microfossils that may possess a multilayered wall ultrastructure and/or ornamented surfaces[14,61,62] (Fig. 2g, i). Microtunnels can form through the activity of endolithic organisms that bore into rocks such as basalt or carbonates, which produces ichnofossils[23,24]. However, it is difficult to demonstrate the biogenicity of these tunnels, because they resemble tubes that form abiotically by pyrite migration within rocks[63] or by displacement of other minerals or organics during metamorphism[24,25]. A complementary approach is to artificially fossilize microorganisms by mineralization or by letting them decay naturally in the laboratory, to improve our understanding of the fossilization processes and to better interpret the fossil record. This idea itself is not new[64,65] but in the past decade experiments and high-resolution analytical techniques have examined the effects of mineralization, diagenesis and metamorphism on microbial cells[32,66–70]. Despite the challenge of reproducing natural conditions, these experimental approaches have explored the physicochemical conditions under which morphological and chemical biological information are retained or altered, and under which pseudosignatures can form. They help to discriminate true life signatures from abiotic products that may also be preserved (sometimes even co-occurring within the same rock)[14].

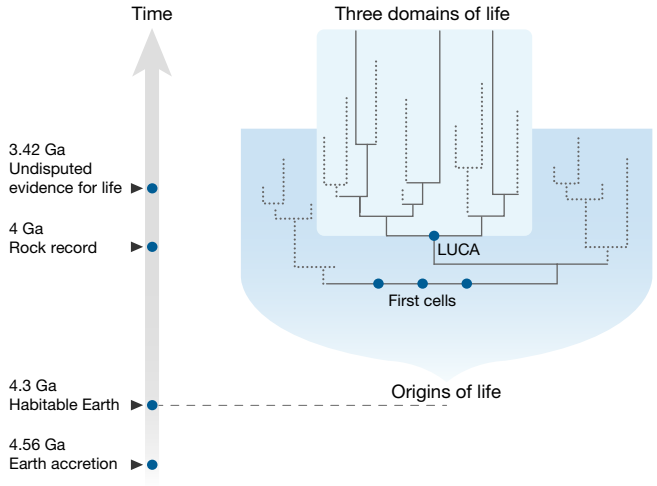

Fig. 3 | Placing biosignatures on the tree of life. Placing biosignatures within the three domains of life, before the last universal common ancestor (LUCA) or in extinct clades within or parallel to the three extant domains can be a challenge, owing to the difficulty of identifying them taxonomically, the occurrence of lateral gene transfer, our incomplete knowledge of microbiology and the incompleteness of the rock and fossil records.

Evidencing biogenicity

We might then ask how we can be confident that a purported trace of life is indeed biogenic. The answer lies in the combination of evidence available, and the careful characterization of the geological context and fossilization processes that are crucial for interpreting this evidence, combined with a lack of abiotic explanations for the observations. In the earliest rock record, the evidence rarely is fully conclusive (because of poor preservation) but instead ranges—with increasing confidence—from putative to possible to probable biosignatures. Deciphering the biogenicity of an object or carbonaceous material is very difficult, even when using cutting-edge in situ techniques. This emphasizes the difficulties of finding unambiguous traces of ancient life on other planets without being able to bring samples back to Earth[71], but also helps to constrain the best targets for preserving possible biosignatures (such as mudstones and shales[72]) when designing sampling strategies for Mars missions[73].

Characterizing the structure, and molecular, elemental and isotopic composition, of ancient carbonaceous matter may give some clues to its origin[3,10,14,27,52,74,75] and possible contamination by younger molecules[3,13,27], but these techniques are not sufficient to determine the biogenicity of this matter even when using advanced analytical techniques. Other necessary co-occurring sources of evidence include the co-variation of other isotopes, the N/C ratio[32,75], the presence of microfossils[21,76], stromatolites[19,77–80] or microbial mat structures[74,81], and the geological context being plausible for life habitat and preservation. Notably, these observations should not be explicable through abiotic processes.

For simple life forms, morphology alone is not sufficient for determining biogenicity, but instead needs to be combined with studies of populations with biological size ranges, observations of cellular division, as well as a hollow structure with continuous carbonaceous cell walls. The presence of organic molecules or a metallome that is indicative of biochemistry, or degradation patterns of decaying, flattened and folded cells, and a distribution that shows fossilized behaviour (orientation and distribution within the rock, caused by mobility and interaction with the environment) may also support a biological origin[14,19,20,77,82]. The strongest confidence in biogenicity can be reached for Proterozoic eukaryotes that show morphological complexity (Fig. 2g, i). A high confidence for biogenicity can also be achieved for prokaryotes (Fig. 2c–e, h) and (as-yet-unidentified) hollow, organic-walled microfossils with simple morphologies (Fig. 1d–j), when these are preserved as carbonaceous compressions in fine-grained sediments[21]. The challenge increases in lithologies such as chert. Impregnation by silica-rich fluids (silicification) may lead to exquisite three-dimensional preservation of microfossils in rocks of the Proterozoic eon (and younger rock record) (Fig. 2d, e), such as in the iconic 1.9-Ga Gunflint stromatolitic chert[76,83] in Canada (Fig. 2d). The interpretation of microstructures preserved in older cherts of the Archaean eon poses more problems, owing to the simpler morphologies of early putative microfossils. Chert forms by the precipitation of silica gel and crystallization of quartz minerals, or by the silicification of pre-existing rocks by hydrothermal fluids. Under such conditions, abiotic or biological organic molecules may come to coat[15,26,84] mineral grains (Fig. 1g) or volcanic particles (tephra)[49,55,85] (Fig. 1h), and may have isotopic and kerogenous chemistries that are similar to those of life. Detailed microscopic analyses may help to discriminate between pseudofossils and true microfossils within these assemblages: pseudofossils may show discontinuous organic walls rather than continuous kerogenous cell walls; walls that are granular, or thicker than biological cell walls; and a size frequency distribution with a large standard deviation[55,59], unlike the size frequency distributions of prokaryotic populations. However, a large size distribution may also reflect degradational gradients[59] or plurimodality, such as the presence of several strains, the life stages of a single strain or various colony sizes.

Challenges in identification

Once the biogenicity of morphological or chemical signatures (as well as their endogenicity and the syngenicity) is established, determining the palaeobiology and identity of these traces of life requires an additional range of observations.

A key question is how these signatures can be interpreted after processes have altered or erased part of the original biological information. Most geochemical and morphological traces are compared to, and interpreted in terms of, modern metabolisms or microorganisms. However, these metabolisms and organisms have evolved. It is also possible that the early signatures might have been produced by alternative biochemical pathways in organisms that existed before the three extant domains of life (Bacteria, Archaea and Eukaryota) diverged—that is, before the last universal common ancestor of the three domains of life—or in extinct organisms that paralleled the evolution of these domains (Fig. 3). Some signatures might also be the result of lateral gene transfer between contemporaneous and unrelated organisms within and outside the three domains: in summary, we should consider the limits of actualism in interpreting early putative traces of life.

We must also bear in mind that the diversity of life is far from constrained within the three domains[86–88]. Moreover, current understandings of modern microbial life and its taxonomically informative characters or metabolic signatures, in which chemical and morphological convergences are known to exist, even between domains, are still incomplete. In other words, what are the limits of our knowledge in microbiology?

Palaeontologists have to rely on information other than the genome and internal cellular organization to identify the biological affinities of early microfossils. In some cases, the morphology, mode of division, presence of ornamentation, ultrastructure and chemistry of carbonaceous cell walls—when combined with the distribution pattern within the hosting rocks and the characteristics of the preservational environment—may help in deciphering the identity of microfossils[14,15,61,62,76,82,89]. However, most early cellular fossils remain unidentified because they lack taxonomically informative characters, and therefore we cannot relate them to an extant clade (Fig. 3). Moreover, these fossils may record traces of extinct lineages that have no modern counterpart[21,52]. Several techniques that are applicable to nano- to microscale organic or mineral samples can be used to characterize the chemistry of these organisms[3,10], and even have the potential to reveal biomolecules that are specific to extant clades as well as isotopic and metal compositions that are indicative of metabolism. Even if the fossils themselves remain unidentified, their morphological or chemical

attributes may provide evidence for biological innovations and the evolution of biochemical pathways that is useful for reconstructing the major steps in the evolution of life[14,15].

The earliest record of life

Even if Earth has probably had habitable conditions for 4.3 billion years, strong evidence must support claims for the presence of early traces of life and abiotic explanations for observations must be considered[12,20]. The oldest record of organic matter with a carbon isotope composition that is consistent with life processes has been reported in 4.1-Ga zircons[90], in 3.95-Ga metamorphic rocks from Canada[91], and in the 3.8–3.7-Ga Akilia belt[92] and Isua belt[92–95] in Greenland. As explained above, the presence of carbonaceous material and the carbon isotopic composition do not constitute sufficient criteria for establishing biogenicity, and the geological context or antiquity of this organic matter has often been debated[3,7,96–99]. Based on carbon and iron isotopes, iron metabolism has previously been inferred in the 3.8-Ga Banded Iron Formation from Isua[100,101], but these isotopes can also be altered or fractionated abiotically[39,102]. Unfortunately, microfossils or microbial mats (which would strengthen claims of biogenicity) are unlikely to be found in these highly metamorphosed rocks, even in less-disputed deep-sea graphitic metasediments[94,95]. Early claims of microfossils in 3.7-Ga Isua metamorphic rocks[103] have subsequently been reassessed as fluid inclusions[104] or contamination by younger microorganisms boring into and fossilized in the rock[9]. Convex-up structures from Isua that have more recently been interpreted as the oldest stromatolites[105] are more likely to represent deformation structures[4,42], because they show convex-down next to convex-up morphologies and occur in tightly folded rocks with a questionable original lithology. Mineral tubes of iron oxide from 3.7-Ga rocks from Canada—originally interpreted as putative iron-oxidizing bacteria preserved in precipitates related to seafloor hydrothermal vents[106]—have been reassessed as volcanic glass[55], and have diameters larger than those of modern iron-oxidizing bacteria. Traces in 3.47-Ga pillow lavas that have been interpreted as endolithic burrows[23] can be formed abiotically as titanite tubes of metamorphic origin[24], or as ambient inclusions trails that are associated with the displacement of mineral and/or organic inclusions[25].

Better-preserved (that is, less-metamorphosed) rocks occur in younger Archean cratons, such as the 3.5–3.0-Ga Pilbara craton of Western Australia and the Barberton region of South Africa and Swaziland. The 3.48-Ga Dresser Formation (Australia) was an active volcanic environment, with intense hydrothermal fluid circulation and frequent eruptions[107]. These rocks preserve putative stromatolites[44,108], microfossils[8,20,109] and sulfur[110–113] and carbon[36,114] isotopic compositions, but most of these have been questioned[3,8,43,45,49,99] on the grounds of possible abiotic origins, hydrothermal alteration, a lack of supporting geochemical evidence, the solid rather than hollow nature of their putative filaments, or poor preservation. Among these putative forms of evidence, the biological origin of the sulfur isotopic composition strongly relies on the disputed origin of the minerals in which it is measured, which is important for discarding abiotic high-temperature fractionation as an explanation[8,115], despite the use of quadruple sulfur isotopes[8,40]. Different texture-specific carbon-isotope compositions in carbonaceous material present in disseminated clots and in fine laminae preserved in chert[36] have been interpreted as biological, on the basis of this systematic trend and the absence of evidence for hydrocarbon migration. However, this carbonaceous material is not directly associated with microfossils or unambiguous microbial mats and stromatolites, which would help to strengthen claims for its biogenicity. Heterogeneous sulfur-isotope compositions of pyrite[112] associated with putative pyritized organic mat layers have been interpreted as microbial, although a thermochemical origin could not be completely discounted. Recently, laminated convex-up precipitates (which have been interpreted as stromatolites with possible gas bubbles and putative microbial fabrics) have been reported in terrestrial hot-spring deposits (geyserites)[116], but they are not associated with microfossils and/or carbonaceous material with an isotopic composition that would support a biological origin. Geochemical data are also needed for some of the possible stromatolites that preserve microtextures[45]. In summary, the Dresser Formation includes a variety of putative or possible morphological and geochemical biosignatures, and is potentially one of the earliest microbial ecosystems that is preserved on Earth. Further study might help to discard abiotic hypotheses, which would push back or reach the limits of confidence in information that can be retrieved from very old rocks.

Filamentous microstructures associated with carbonaceous matter enriched in light carbon isotopes in the 3.45-Ga Apex chert Formation (Australia) were initially interpreted as microfossils[50]. These microstructures were subsequently reconsidered as abiotic pseudofossils, because their morphology was inconsistent with biology and was more compatible with fluid migration around minerals or mineral artefacts[48,117,118] and because of a re-evaluation of their geological context as hydrothermal rather than marine shallow water[84]. The controversy regarding the origin of carbonaceous material and possible traces of life in various locations and chert types of the Apex Formation continues, despite several events of hydrothermal overprinting of the whole succession and the use of high-resolution techniques and recent field sampling by multiple laboratories[27,51,59,119,120]. Recently, possible microbial mat structures that consist of wavy organic layers that trap grain sediments have been reported in this iconic locality[120].

Putative microfossils and diverse stromatolites[5,121,122] have been reported in the 3.5–3.25-Ga Onverwacht and Fig Tree Groups (Barberton, South Africa). The more convincing of these are conical stromatolites that preserve microbial microtextures in the 2.98-Ga Pongola Supergroup (South Africa) (see discussion and references in refs [5,11]). Filamentous, coccoidal and lenticular putative microfossils have been described from the 3.434–3.416-Ga Kromberg Formation (South Africa)[8,53,123,124] but have been reconsidered as abiotic, owing to evidence of fluid migration or similarities with volcanic particles[8,26,55,85]. More-recent analyses of kerogenous lenticular forms from the Kromberg Formation have revealed carbon-isotope compositions that are consistent with biology[124], but ultrastructural studies would help to support claims of biogenicity. Geological context, petrography and kerogen geochemistry support a microbial origin for microbial mat structures and ripped-up fragments from the 3.33-Ga Josefdal chert[74] and the 3.416-Ga Buck Reef chert[125], respectively. Probable silicified microbial mat structures reported in the 3.47-Ga Hooggenoeg Formation[85] need in situ geochemical analyses to confirm their biogenicity.

One of the strongest cases that has so far been documented for early traces of life is the stromatolites of the 3.426–3.35-Ga Strelley Pool Formation (Australia)[19,41,108,126–128]. Their biogenicity was proposed and then questioned[43,129], but the improved characterization of their geological context (which is plausible for life, being a marine shallow-water hypersaline evaporitic carbonate platform[19,130]) and detailed multiscale studies have provided a combination of evidence that supports their biological origin—albeit perhaps less strongly for stromatolites without preserved organic matter[131]. These stromatolites occur as seven types of three-dimensional convex-up structures that display a mostly wavy, but sometimes more complex, branching or conical morphology. The most compelling evidence for biogenicity are these branching and conical stromatolites with laminae of variable thicknesses (non-isopachous) that are laterally linked by flat laminated carbonates; so far, it has not been possible to explain these features as the result of abiotic processes[8,11]. These morphologies vary along a carbonate platform that is several kilometres long, as a function of hydrodynamic conditions and water depth. This pattern repeats itself on different platforms as well as vertically in response to sea-level change, which is suggestive of microbial reef ecology[19]. Some of the domal stromatolites preserve organic laminae[19] that have yielded carbon- and sulfur-isotope signatures and nitrogen and sulfur elements preserved in carbonaceous particles, indicating a chemistry that is consistent with multiple microbial metabolisms[131–133]. Organic material is not preserved in all types of stromatolite but, when it is, the

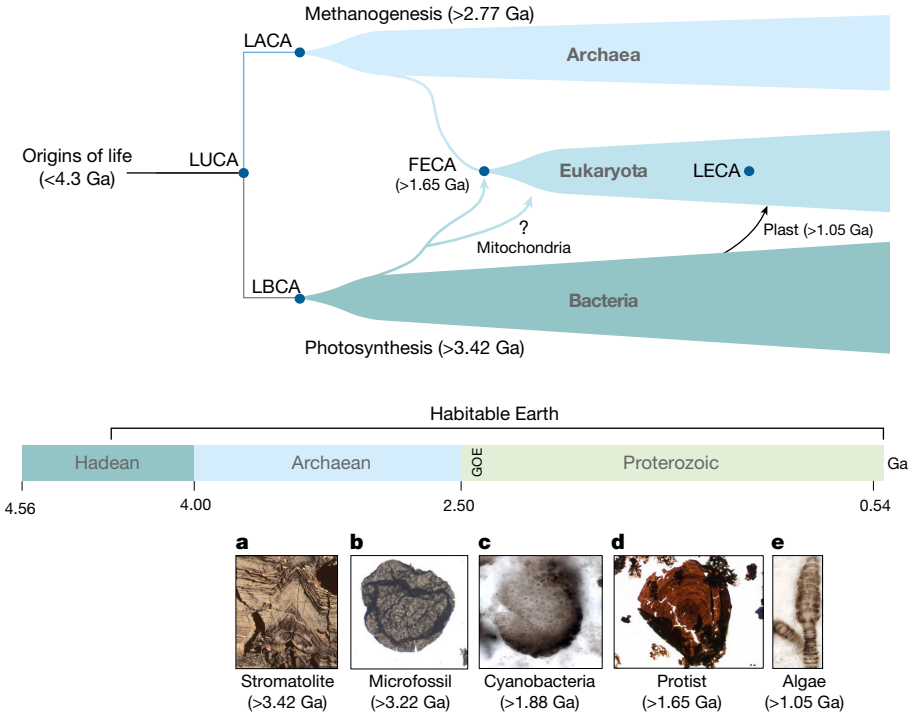

Fig. 4 | Evolution of early life. Morphological and chemical biosignatures provide constraints on the minimum ages of the three domains of life, early metabolisms and biological innovations. Bacteria date back to at least 3.42 Ga, and Archaea to at least 2.77 Ga, which implies a last Bacteria common ancestor (LBCA) and a last Archaea common ancestor (LACA) older than these dates, respectively, and a last universal common ancestor earlier than this, preceded by the origin of life—all after 4.3 Ga, at which point the Earth became habitable. Eukaryotes emerged from prokaryotic ancestors before 1.65 Ga (the minimum age of the oldest eukaryotic microfossils), which implies a first Eukaryota common ancestor (FECA) older than this date. Endosymbiotic events were important in the evolution of early eukaryotes, including the origin of the mitochondria and the chloroplast from proteobacterial and cyanobacterial ancestors, respectively. Fossil algae suggest that the acquisition of plasts dates back to more than 1.05 Ga, which implies a last Eukaryota common ancestor (LECA) that is older than this date. GOE, Great Oxidation Event. **a**, Conical stromatolite (3.426–3.35-Ga Strelley Pool Formation, Australia). **b**, Organic-walled microfossil (acritarch), 220 μm in diameter (3.22-Ga Moodies Group, South Africa). **c**, *Eoentophysallis*, the oldest undisputed colonial cyanobacterial microfossil (1.88-Ga Belcher Group, Canada). **d**, *Valeria lophostriata*, 400 μm in diameter; the oldest fossil protist with an organic wall ornamented with concentric striations (>1.65-Ga Mallapunyah Formation, Australia). **e**, *Bangiomorpha pubescens*, 200-μm long; an undisputed benthic multicellular red algae (1.05-Ga Hunting Formation, Canada). Image in **a** reproduced from ref. [129] with permission; images by E.J.J. (**b**, **d**) or courtesy of C. Demoulin (**c**) or N. Butterfield (**e**).

non-random distribution of this material in the domes or convex-up laminae suggests a concentration of organic biopolymers on stromatolite slopes that could serve as a template for the passive precipitation and trapping of minerals. Such criteria, when combined with the morphological complexity of the stromatolites, have previously been used as evidence for the biogenicity of 2.77-Ga stromatolites[134]. The carbonate laminae show lateral variations in their thickness, microscopic textures that differ between and in the cones, and cannot be traced laterally over hundreds of metres of outcrop, contrary to abiotic mineral precipitates[19,47]. If stromatolites can be used as a proxy for autotrophic metabolism such as anoxygenic or oxygenic photosynthesis (although their upward growth might be linked to competition for nutrients rather than, or in addition to, light[11]), then the stromatolites of 3.426–3.35-Ga Strelley Pool Formation provide a minimum age for the evolution of the domain Bacteria, within which this metabolism originated. This implies an earlier date for the last (most recent) common ancestor of Bacteria, an even older last universal common ancestor—and thus an earlier origin of life (Fig. 4).

Bedded cherts in the Strelley Pool Formation, as well as the younger 3.0-Ga Farrel quartzite chert (Australia)[22,52,135], preserve possible microfossil assemblages that include simple aggregates of spheroids and organic films that are possibly abiotic, and abundant large, flanged lenticular carbonaceous microstructures that are more difficult to explain by abiotic processes[49]. Abiotic lenticular volcanic structures can be solitary or form chains with their long axes aligned[49,54], and can presumably be wrapped or filled in by organics[49], which perhaps explains some of the hollow lenses with organic lining that are observed in situ in chert of the Strelley Pool Formation[52]. However, some of the chains of lenses in the Strelley Pool Formation are entirely kerogenous, keep their integrity and stay attached after extraction using acid demineralization, which represent observations that are more difficult to explain abiotically[49,52,55,59]. When extracted from the chert, transmission electron microscopy analyses show an alveolar structure[52], unlike a single hollow cell or an empty colonial envelope. Associated N/C ratios, elemental maps[135] and texture-specific carbon[136,137] and nitrogen[138] isotopic heterogeneities in the microstructure assemblages of the Strelley Pool Formation and Farrel quartzite may provide further support for a biological origin of the kerogen, but not necessarily of the microstructures. These heterogeneous isotopic features of the microstructure assemblages may also reflect several generations of organic matter or multispecies biofilms from distinct precursors with different isotopic compositions[49]. It is also possible that some of the flanged kerogenous lenses (with an alveolar ultrastructure) could have been bacterial colonies that were growing in Archaean silica-rich (gel-like) water. Notably, laboratory experiments and theoretical physics have shown how the morphology and size of bacterial colonies growing within a three-dimensional gel matrix change from diffuse and circular to compact and lenticular at higher concentrations of agar concentration, owing to mechanical forces[139]. These experiments have also documented how a monolayered circular two-dimensional colony changes into a three-dimensional colony with a multilayered, thicker central part and a monolayered outer ring (or flange)[140]. Further ultrastructural analyses of extracted lenses, and laboratory experiments testing these hypotheses—such as encapsulation or filling of vesicular volcanic sediments by abiotic organics or biofilms[49] or bacterial colony growth in silica gel, and artificial fossilization of these—might help

to discriminate between possibly co-occurring abiotic and biological microstructures.

Beside shallow-water carbonate and volcanic settings, siliciclastic environments can also be colonized by microbial mats, stabilizing intertidal sand or colonizing rock cavities or surfaces, and preserved in situ by silicification or ripped-up and rolled-up by waves or currents. A combination of criteria has been proposed to discriminate organic-rich microbial mat laminations from abiotic accumulation of organics by pressure-solution fronts or fluid migration[16,74,81,85,141]. These mat layers should occur as sets of fine, crinkly, organic laminae of variable thickness (non-isopachous), which drape detrital grains that are oriented parallel to the laminae and are 'floating' without grain-to-grain contact. They should be preserved in a geological context that is plausible for life, have an isotopic composition that is consistent with biology, co-occur with ripped- and rolled-up fragments eroded from the mat (indicating the flexibility of a soft, but coherent, sediment layer that is bound by microbial organic matter) and have a particular pattern of orientation. Cases of such microbial mats are reported in the rock record from 3.47 Ga, in the Barberton area (South Africa) and the Pilbara (Australia)[16,81,85,125,141] (although some need confirmation of biogenicity with in situ geochemical analyses). While some of these mat builders have been interpreted as cyanobacteria (on the basis of comparisons with modern analogues), there is no firm evidence so far that supports oxygenic photosynthesis over other autotrophic and heterotrophic metabolisms[82]. Collectively, these traces—when combined with multiple other biosignatures—indicate that benthic microbial mats colonized marine shallow water, volcanic islands and fluviatile sediments on sub-aerially exposed continental crust early on.

In the 3.22-Ga Moodies Group (Barberton Greenstone Belt, South Africa), populations of large organic-walled, spheroidal microfossils are preserved as carbonaceous compressions that are flattened parallel to bedding in tidal shales and siltstones[21]; these features supports their syngenicity, which is also indicated by Raman spectroscopy. These microfosssils show a continuous and recalcitrant kerogenous cell wall with a carbon isotopic chemistry and degradational features (folding and wrinkling) that are consistent with biology, are hollow and survive acid extraction from the host rock. To our current knowledge, no abiotic processes can reproduce similar structures in such a context, which strengthens the hypothesis of their biogenicity[15,21,142]. Future investigations might lead to the discovery of other occurrences and further clues to their identity. The Moodies Group also preserves other traces of life, such as extensive microbial mats in shallow-marine tidal deposits[141] and terrestrial deposits[81], in which they drape fluvial conglomerates and gravelly sandstones and are associated with kerogen with carbon, nitrogen and sulfur isotopic compositions that are consistent with microbial metabolisms[143,144]. The identity of the Moodies microfossils—whether prokaryotic, eukaryotic or even another extinct form of life—is unknown. Nevertheless, these microfossils indicate the evolution of large benthic or planktonic cells or colonial envelopes, which synthesized recalcitrant organic biopolymers and lived in marine shallow water[21] not far from microbial mats. The age of the domain Eukaryota is unconstrained and stem eukaryotes could presumably be as old as the Archaean eon, but this hypothesis is difficult to test in the absence of diagnostic fossil molecules or microfossils with a morphological complexity that is unknown in prokaryotes. To date, the oldest unambiguous traces of eukaryotes are hollow microfossils with ornamented organic walls that are preserved in 1.65-Ga shales from Australia[62] (Fig. 4) and China[145].

The Archaea have a unique metabolism, methanogenesis, that can be evidenced by strongly negative fractionations of carbon isotopes in kerogen, which are reported worldwide between 2.8 and 2.6 Ga[6,79,93], including in the stromatolites of the lacustrine 2.77–2.72-Ga Fortescue Group[78,79], perhaps in the 3.42–3.35-Ga Strelley Pool Formation (for which other metabolisms are possible)[132] and (debatably) in 3.48-Ga hydrothermally derived methane-bearing fluid inclusions[99,114]. Indirectly, the oldest fossil traces of eukaryotes at 1.65 Ga[62,145] provide a minimum age for the domain Archaea (although not necessarily for methanogenic Archaea) (Fig. 4), because eukaryotes are thought to originate from an archaeal ancestor[88].

Despite important challenges, the oldest unambiguous traces of life preserved on Earth thus firmly support the presence of a microbial biosphere in terrestrial habitats since at least 3.2 Ga and in marine settings since at least 3.42 Ga, and perhaps earlier (Fig. 4). Convincing data provide constraints on the minimum ages of the three domains of life (Bacteria, more than 3.42 Ga; Archaea, more than 2.77 Ga; and Eukaryota, more than 1.65 Ga), which suggests an origin for these domains earlier than these respective dates. These data also document the early evolution of specific metabolisms and other biological innovations, as well as unidentified forms of cellular life and microbial mats.

Perspectives

Among the challenges discussed here, three in particular limit our understanding of traces of early life and require additional fundamental research: (1) the abiotic processes that mimic biological morphologies and chemistries; (2) our incomplete knowledge of fundamental microbiology, such as the morphology, molecular composition, isotopic composition, metallome, ultrastructure of preservable cellular components, and the diversity, metabolisms and phylogeny of microbial life; and (3) the effects of fossilization processes (that is, taphonomy—how morphological and chemical traces get preserved and altered). The diversity and resolution of techniques available for characterizing possible biosignatures are now impressive, but are only meaningful if interpreted in their micro- and macroscale geological contexts and in combination with other approaches. As research and analytic development progresses, newly discovered and previously known geological sites—including those presented here, as well as many others—can be investigated or reinvestigated, and their possible signatures of life can be assessed with a greater confidence, to the limits of their preservation. The characterization of reliable biosignatures for microbial life is crucial for understanding the early evolution of the biosphere of the Earth. We can then address questions regarding the conditions for the appearance and development of life on other planetary bodies (habitability), and the probability for an extraterrestrial biosphere to develop complex metabolisms, such as anoxygenic or oxygenic photosynthesis, or morphologies, such as complex multicellularity. This research is critical for the advancement of life-detection strategies, instruments and missions that are applicable to other planets of the solar system and to the atmospheres of rocky exoplanets, as space agencies have recently come to appreciate.

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