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Molecular fossils from organically preserved Ediacara biota reveal cyanobacterial origin for *Beltanelliformis*

Ilya Bobrovskiy[1], Janet M. Hope[1], Anna Krasnova[2,3], Andrey Ivantsov[2] and Jochen J. Brocks[1*]

The Ediacara biota (~575–541 million years ago) mark the emergence of large, complex organisms in the palaeontological record, preluding the radiation of modern animal phyla. However, their phylogenetic relationships, even at the domain level, remain controversial. We report the discovery of molecular fossils from organically preserved specimens of *Beltanelliformis*, demonstrating that they represent large spherical colonies of cyanobacteria. The conservation of molecular remains in organically preserved Ediacaran organisms opens a new path for unravelling the natures of the Ediacara biota.

Beltanelliformis is one of the most revisited Ediacaran macrofossils. It comprises *Nemiana* and *Beltanelloides* taphonomic forms. *Nemiana* are commonly preserved at the base of sandstone lenses as circular, convex tubercles with or without concentric folds, while *Beltanelloides* are found within clay or carbonate as low-relief circular imprints with concentric folds near the edges[1]. *Nemiana* were initially described as abiological structures or compared with jellyfish bodies[2]. Later, *Nemiana* were interpreted as the internal sand skeletons of corals[3] or benthic demosponges that agglutinated sand to support their bodies[4]. *Beltanelloides* were described as benthic[5] or planktonic[4] eukaryotic algae or as fungal colonies with concentric folds caused by non-uniform growth[6]. Some studies regarded both taphonomic forms of *Beltanelliformis* as cnidarian resting traces based on their similarity to Devonian anemone burrows *Alpertia santacrucensis*, interpreting the concentric folds as musculature traces[7]. In contrast, some other studies found that *Beltanelliformis* do not show any characteristics typical of cnidarians and lack the typical structures of algae and should, thus, be interpreted as colonial prokaryotes[8–10]. Others found that interpretation of *Beltanelliformis* as fluid-filled vesicles with a firm and flexible organic wall is inconsistent with both cnidarian and prokaryotic interpretations, and that these characteristics point to benthic green algal gametophytes instead[11,12].

The specimens of *Beltanelliformis* studied here were collected from ~558-million-year-old Ediacaran deposits at the Lyamtsa locality, White Sea, Russia that formed in shallow marine environments within the photic zone[13]. *Beltanelliformis* are preserved within clay as low-relief, slightly convex, circular imprints up to 1.5 cm in diameter with concentric folds at the edges and covered by a thin, translucent film of organic matter (Fig. 1b and Supplementary Fig. 1). The organic film is restricted to *Beltanelliformis* surfaces and is not found beyond and between the fossils. Thus, it does not represent a film that covered the entire sediment surface. Thicker, brown-coloured organic matter, probably consisting of macroalgae (see Supplementary Information), is preserved on the same surface next to *Beltanelliformis* (Fig. 1a). We separately detached the organic matter of *Beltanelliformis* and macroalgae from the rock surface and extracted hydrocarbon biomarkers under strict exclusion of contamination. Biomarkers were analysed by gas chromatography-mass spectrometry (see Methods) and were found to represent the best-preserved molecular remains of Precambrian age to date.

The organic films of *Beltanelliformis* and macroalgae, preserved adjacent to each other on the same clay surface, must have experienced similar diagenetic and burial conditions and comparable degrees of microbial reworking. Differences in the molecular content of these films can, thus, largely be ascribed to the source organisms. In the macroalgal organic matter, steranes (S), the molecular fossils of eukaryotic membrane sterols, dramatically dominate over bacterial hopanes (H) (H/S = 0.061; Table 1 and Fig. 1c), confirming the eukaryotic origin of the film. Steranes exhibit a very high C_{29} predominance (C_{27}:C_{28}:C_{29} = 8%:5%:87%), which is typical of Chlorophyta (green algae) and diagnostic for bitumens of Ediacaran age[14], confirming the indigenous nature of the hydrocarbons. The distribution of *n*-alkanes in the macroalgal film is unique for the Precambrian (Fig. 1e). The short-chain *n*-alkanes ($C_{18–22}$) exhibit a slight odd-over-even carbon number predominance (odd-to-even predominance index $(OEP)_{18–22}$ = 1.30; Table 1), but their abundances are insignificant relative to long-chain homologues ($C_{23–33}$). The long-chain alkanes do not possess any carbon number preference ($OEP_{25–29}$ = 0.98) and are probably the diagenetic products of functionalized biomolecules, such as long-chain fatty acids or alcohols[15]. Suitable precursors are well known from microalgae[16,17], although their presence in macroalgae is less well constrained[18]. Long-chain fatty acids might have accumulated in the algal film due to preferential bacterial degradation of the short-chain homologues[16], or might have been protected from degradation as part of a cuticle layer or biopolymer such as algaenan, which is widespread among chlorophytes[19].

The biomarkers extracted from organically preserved *Beltanelliformis* are remarkably distinct from the macroalgae despite the close physical association (Fig. 1 and Table 1). The hopane-to-sterane ratio is 60 times higher than in the algal film (H/S = 3.6), indicating that the source is largely bacterial. To our knowledge, such a robust contrast in bacterial and eukaryotic marker abundances in adjacent samples has never been observed before. Steranes are present in the *Beltanelliformis* extract, but show the same unusual C_{29} predominance as the adjacent macroalgal film, pointing to minor

[1]Research School of Earth Sciences, Australian National University, Canberra, Australian Capital Territory, Australia. [2]Borissiak Paleontological Institute, Russian Academy of Sciences, Moscow, Russia. [3]Faculty of Geology, Lomonosov Moscow State University, Moscow, Russia.
*e-mail: jochen.brocks@anu.edu.au

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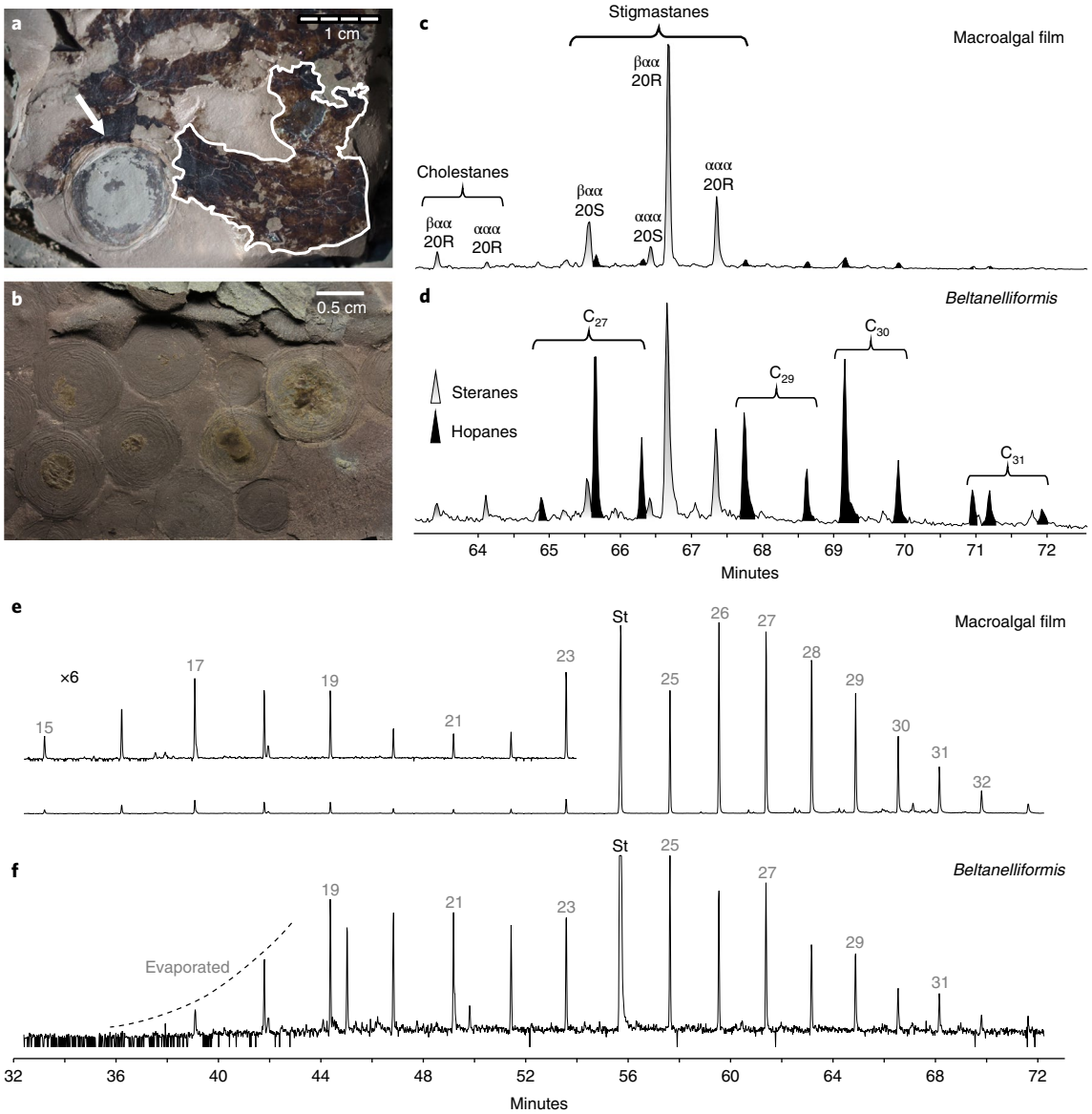

Fig. 1 | Distribution of steranes, hopanes and *n*-alkanes in the extracts of a macroalgal film and *Beltanelliformis*. **a**, Macroalgal film preserved next to *Beltanelliformis*. The white arrow points at an overlap between *Beltanelliformis* and the macroalgal film. The white outline highlights the extracted algal surface. The whole scale bar is 1 cm. **b**, Organically preserved *Beltanelliformis*. The whole scale bar is 0.5 cm, each unit is 1 mm. **c**, Metastable reaction monitoring chromatogram showing the sum of C_{27-31} hopane and C_{27-29} sterane traces of the macroalgal film extract. **d**, Metastable reaction monitoring chromatogram showing the sum of C_{27-31} hopane and C_{27-29} sterane traces of the *Beltanelliformis* extract. C_x denotes the carbon number of hopanes (for isomer identification, see Supplementary Fig. 5). ααα is the 5α(H),14α(H),17α(H) sterane isomer and βαα is the 5β(H),14α(H),17α(H) sterane isomer. **e**, Distribution of *n*-alkanes in the macroalgal film extract on the *m/z* 85 trace. **f**, Distribution of *n*-alkanes in the *Beltanelliformis* extracts on the *m/z* 85 trace. The numbers in **e** and **f** indicate the carbon number of *n*-alkanes. In the inset of **e**, ×6 signifies 6 times magnification of the chromatogram. St is the 18-methyleicosanoic acid methylester internal standard. Credit: **b**, S. Bagirov.

physical overlap of *Beltanelliformis* and algal organic matter (for example, Fig. 1a).

The main source of hopanoids, the biogenic precursors of hopanes, are aerobic alphaproteobacteria and oxygenic cyanobacteria[20]. Some anaerobes and microaerophiles also have the capacity to biosynthesize hopanols, including sulphate-reducing bacteria and methylotrophs[21], and these may have contributed hopanes during microbial degradation of *Beltanelliformis*. However, the low content of hopanes relative to steranes in the macroalgal film indicates that this heterotrophic contribution must have been minor. Evidence of oxic bottom waters in the Ediacaran sea in the Lyamtsa locality comes from abundant benthic macroalgae, trace fossils and burrows[22], which makes aerobic colonies of heterotrophic or phototrophic bacteria the most likely candidates for *Beltanelliformis*.

Further evidence about the nature of *Beltanelliformis* comes from the distribution of *n*-alkanes. In contrast with the algal film, *Beltanelliformis* exhibits *n*-alkanes with OEP at all chain lengths ($OEP_{18-22} = 1.40$, $OEP_{25-29} = 1.21$; Table 1), and short and long-chained homologues are nearly equally abundant (Fig. 1f). The preservation of such a distinct OEP strongly suggests that the precursors included biological long-chain *n*-alkanes or *n*-alkenes, biosynthetic hydrocarbons without additional functional groups[15]. Among bacteria, only cyanobacteria are known to produce such long-chain hydrocarbons with OEP[16,23–25], which makes

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Table 1 | Biomarker ratios for organic film and clay extracts

	Beltanelliformis	Macroalgal film	*Vendotaenides*	Clay	Cv[a]
H/S[b]	3.6	0.061	0.69	4.7	0.86%
C_{27}:C_{28}:C_{29} steranes	7%:5%:88%	8%:4%:88%	13%:3%:84%	11%:8%:81%	3.02%:3.93%:0.43%
βαα/αααα C_{29} steranes	3.0	3.7	2.0	0.47	6.97%
βα/(βα + αβ) C_{30} hopanes	0.26	0.26	0.25	0.25	1.39%
Ts/(Ts + Tm) C_{27} hopanes	0.082	0.071	0.079	0.080	2.45%
C_{30}/C_{31} hopanes[c]	2.56	3.06	2.66	1.51	3.89%
C_{29}/C_{30} hopanes[c]	0.70	0.87	0.86	0.73	3.84%
2-MHI[d]	0.85%	1.28%	ND	0.68%	5.4%
OEP_{18-22}[e]	1.40	1.30	1.27	1.10	1.88%
OEP_{25-29}[e]	1.21	0.98	0.99	1.35	0.84%

[a]Coefficient of variation (%). [b]H/S = Σ(C_{27-35} hopanes)/Σ(C_{27-29} steranes); hopanes: C_{27} = Σ(Ts, Tm, β), C_{29} = Σ(αβ, Ts, βα), C_{30} = Σ(αβ, βα), C_{31-35} = Σ(αβ-22(S + R), βα), αβ = 17α(H)21β(H), βα = 17β(H)21α(H); steranes: C_{27} = Σ(βα-20(S + R)-diacholestane, αααα- and βααα-20(S + R)-cholestane), C_{28} = Σ(βα-20(S + R)-diaergostane, αααα- and βααα-20(S + R)-ergostane), C_{29} = Σ(βα-20(S + R)-diastigmastane, αααα- and βααα-20(S + R)-stigmastane), αααα = 5α(H),14α(H),17α(H), βααα = 5β(H),14α(H),17α(H). [c]C_{29} = Σ(αβ, Ts, βα), C_{30} hopane = Σ(αβ, βα), C_{31} = Σ(αβ-22(S + R), βα). [d]2-methylhopane index (%) = 100 × (Σ C_{31} 2α + 2β-methylhopane)/(Σ C_{31} 2α + 2β-methylhopane + Σ(αβ-22(S + R), βα) C_{30} hopane). [e]Odd-to-even predominance index for *n*-alkanes in the carbon number range 18–22 and 25–29, respectively: OEP_{18-22} = ($4C_{19}$ + $4C_{21}$)/(C_{18} + $6C_{20}$ + C_{22}); OEP_{25-29} = (C_{25} + $6C_{27}$ + C_{29})/($4C_{26}$ + $4C_{28}$).ND, components not detected.

a strong case that *Beltanelliformis* represent colonial structures of cyanobacteria. This conclusion is also supported by the distribution of hopane homologues in the *Beltanelliformis* extract (Supplementary Information).

Based on morphology, *Beltanelliformis* can be compared to modern spherical cyanobacterial freshwater colonies of the genus *Nostoc* that possess degradation-resistant outer envelopes (Supplementary Fig. 4)[9]. *Beltanelliformis* may be an early marine representative of such nostocalean colonies, but it may also belong to an extinct taxon.

Among modern organisms, long-chain fatty acids and *n*-alkanes with OEP are abundantly produced by higher plants, where they occur as part of a protective desiccation-resistant cuticle membrane. In some microalgae, long-chain fatty acids were also found to be involved in the formation of the outer cell walls along with aliphatic biopolymers, displaying great similarities with the cuticle layer of higher plants, probably having the same biosynthetic pathway and the same function[17,26]. Interestingly, among modern cyanobacteria, only non-marine forms appear to produce long-chain hydrocarbons[23–25]. This makes it possible that macroalgae and *Beltanelliformis* cyanobacterial colonies from the Ediacaran deposits in the White Sea were partially desiccation resistant and able to survive intermittent subaerial exposure.

Due to the simple morphology of most members of the Ediacara biota and lack of modern analogues for others, there are many controversies about the nature of the Ediacara biota, and the range of interpretations is extremely broad, varying from animals and giant protists to lichen colonies growing on land[27]. Biomarkers add a new dimension to the study of the Ediacara biota, showing here that *Beltanelliformis* were benthic colonial cyanobacteria.

Methods

Samples were collected in pre-baked aluminium foil (300 °C for 9 h) and packed in calico bags under strict avoidance of contamination. Organic matter was removed from the clay surface using a solvent-cleaned scalpel and tweezers. For biomarker analysis, organic matter of *Beltanelliformis* was collected from around 300 specimens from one surface and combined to obtain the best possible signal-to-noise ratio. It was also studied for microstructure on a Zeiss EVO50 scanning electron microscope with an INCA (Energy 350) microanalyser (Oxford Instruments). Hydrocarbons were extracted from the organic matter via ultrasonication (methanol 1 h, dichloromethane for 15 min (×2), dichloromethane:*n*-hexane 1:1 for 15 min). All solvents were 99.9% grade (UltimAR; Mallinckrodt Chemicals).

Extracts from organic films were fractionated into saturated + aromatic and polar fractions. An internal standard, 18-methyleicosanoic acid methylester (Chiron Laboratories AS), was added to the saturated and aromatic fractions, while d4-C_{29}-αααα-ethylcholestane (Chiron Laboratories AS) was added to the saturated hydrocarbon fractions only. The samples were analysed and quantified by gas chromatography-mass spectrometry. A comprehensive, accumulatory system blank was performed covering all analytical steps. For more information about the methods, see the Supplementary Methods.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability. Biomarker raw data are available from the corresponding author upon reasonable request.

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Author contributions

I.B. conceived the study and performed the analyses. I.B. and A.K. collected the samples. J.M.H. helped with the methodology. A.I. provided palaeontological advice. I.B. and J.J.B. interpreted the results and wrote the paper.

Competing interests

The authors declare no competing financial interests.

Additional information

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