Ancient steroids establish the Ediacaran fossil *Dickinsonia* as one of the earliest animals

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The enigmatic Ediacara biota (575 to 541 My) represents the first macroscopic complex organisms in the geological record and may hold the key to our understanding of the origin of animals. Ediacaran macrofossils are "strange as life on another planet" and have evaded taxonomic classification, with interpretations ranging from marine animals or giant single-celled protists to terrestrial lichens. Here we show that lipid biomarkers extracted from organically preserved Ediacaran macrofossils unambiguously clarify their phylogeny. *Dickinsonia* and its relatives solely produced cholesteroids, a hallmark of animals. Our results make these iconic members of the Ediacara biota the oldest confirmed macroscopic animals in the rock record, indicating that the appearance of the Ediacara biota was indeed a prelude to the Cambrian explosion of animal life.

One Sentence Summary: Lipid biomarkers constrain the nature of enigmatic fossil *Dickinsonia* and its morphological relatives, expanding the record of earliest animals.

The Ediacara biota remains one of the greatest mysteries in palaeontology. Members of this assemblage were initially described as animals (*1*, *2*), however, as collections grew, it became apparent that Ediacaran fossils and their body plans are difficult to compare with modern phyla (*3*, *4*). A major complication for the study of Ediacaran organisms is their soft-bodied nature and unique mode of preservation, rarely found in younger fossils. Thus, the interpretation of various members of the Ediacara biota has crossed several Kingdoms and Domains, ranging from bacterial colonies (*5*), marine fungi (*6*), lichens (*7*) and giant protists

(*8, 9*) to stem-group animals and crown-group Eumetazoa (*4, 10, 11*). The recent general consensus is that these fossils are polyphyletic (*12, 13*): at least some members of the Ediacara biota are almost unanimously interpreted as bilaterian animals (*Kimberella*) (*14, 15*), while others are confidently ascribed to giant protozoa (*Palaeopascichnus*) (*16*). *Beltanelliformis*, although previously interpreted as bacteria, benthic and planktonic algae, as well as different animals, is now recognized as a spherical colony of cyanobacteria based on their biomarker content (*17*). The affinity of most other Ediacarans however remains controversial even at the Kingdom level (*4*). Most recently, arguments surrounding these fossils have centred on lichens, giant protists and stem- or crown group Metazoa.

While the lichen hypothesis (*7*) requires an implausible re-interpretation of the habitat of the Ediacara biota from a marine to a continental depositional environment (*18*), for many Ediacaran fossils, including dickinsoniids, it currently seems impossible to distinguish between giant protist and metazoan origins (*4, 19*). Some Ediacaran fossils, such as *Palaeopascichnus*, were likely giant unicellular eukaryotes (protists) (*16*), which means that, in contrast to modern ecosystems, these organisms were present, and sometimes extremely abundant, in shallow-water Ediacaran habitats (*20*). Features of dickinsoniids such as 'quilting' patterns, the inferred absence of dorso-ventral differentiation, and putative external digestion mode were found to be compatible with modern giant protists and hard to reconcile with metazoans (*8, 20*). Some modern giant protists can be up to 25 cm in size (*21*). In the absence of metazoan competition they may have become even larger, thereby possibly providing an explanation for the size range of Ediacaran protistan fossils (*8*). Some giant protists even have a motile lifestyle, compatible with Ediacaran trace fossils (*22*) and dickinsoniid 'footprints' (*15*). For dickinsoniids, the absence of evidence for a mouth and gut, perceived absence of bilateral symmetry and possible external digestion are all consistent with a protistan origin. However, all of the above characteristics are also compatible with basal Metazoa such as the Placozoa that are situated at the very base of Eumetazoa (*23*), while rejection of an external digestion mode and acceptance of supposed cephalization (*15*) may place dickinsoniids even higher on the metazoan tree. The nature of dickinsoniids, and most other Ediacaran fossils, thus remains unresolved.

We applied a novel approach (*17*) to test the lichen, protist and animal hypotheses by studying biomarkers extracted from organically preserved dickinsoniids. Hydrocarbon biomarkers are the molecular fossils of lipids and other biological compounds. Encased in sedimentary rock, biomarkers may retain information about their biological origins for hundreds of millions of years. For instance, hopanes are the hydrocarbon remains of bacterial hopanepolyols, while saturated steranes and aromatic steroids are diagenetic products of eukaryotic sterols. The most common sterols of Eukarya possess a cholesteroid, ergosteroid or stigmasteroid skeleton with 27, 28 or 29 carbon atoms respectively. These C_{27} to C_{29} sterols, distinguished by the alkylation pattern at position C-24 in the sterol side chain, function as membrane modifiers and are widely distributed across extant Eukarya, but their relative abundances can give clues about the source organisms (*24*).

Apart from *Dickinsonia* (Fig. 1B), which is one of the most recognizable Ediacaran fossils, dickinsoniids include *Andiva* (Extended Data Fig. 1C), *Vendia*, *Yorgia* and other flattened Ediacaran organisms with segmented metameric bodies and a median line along the

body axes, separating the 'segments'. The specimens for this study were collected from two surfaces in the Lyamtsa (*Dickinsonia*) and Zimnie Gory (*Andiva)* localities of the Ediacara biota in the White Sea region (Russia). Both *Dickinsonia* and *Andiva* are preserved in negative hyporelief on the sole of sandstones with microbial mat impressions and consist of a thin (up to around 3 μm) film of organic matter. The organic matter was detached from the rock surface (Fig. S1) and extracted for hydrocarbon biomarkers under strict exclusion of contamination (see Methods). Much thinner organic films covering the surfaces around *Andiva* fossils from the Zimnie Gory locality were extracted as well, providing a background signal coming from associated microbial mats. Investigation of biomarker composition of surrounding surfaces and enclosing sedimentary rocks allowed not only to subtract the background signal, but also to make sure that the biomarker signal from the fossils is not contaminated (Supplementary Text) Biomarkers were analysed using gas chromatography-mass spectrometry (see Methods).

The deposits immediately above and below *Dickinsonia* are characterized by a monoaromatic steroid distribution of 10.6–11.9% cholesteroids, 13.4–16.8% ergosteroids and 71.3–76.0% stigmasteroids, consistent with the general steroid distribution of sediments at the Lyamtsa locality (Fig. 1). The strong stigmasteroid predominance is typical for the Ediacaran period and presumably related to green algae (Chlorophyta) inhabiting benthic mats or the water column (*25*). In these and all other Ediacaran sediment samples from the White Sea region, the carbon-number distribution of saturated steranes is nearly identical to the distribution of monoaromatic steroid homologues and always dominated by green algal stigmasteroids (Table 1). In stark contrast, biomarkers extracted from the isolated organic matter of the largest *Dickinsonia* specimen had a monoaromatic steroid distribution of 93% cholesteroids, 1.8% ergosteroids and 5.2% stigmasteroids (Table 1, Fig. 1A). A general trend of increasing monoaromatic cholesteroid abundance from 84.8% to 93.0% from the smaller to the larger *Dickinsonia* specimens (Fig. 1D), reflects decreasing contribution of the green algal background signal (Fig. S2).

The striking abundance of cholesteroids in *Dickinsonia* is corroborated by an unusual sterane isomer distribution. In sediments surrounding the fossils in Lyamtsa and Zimnie Gory localities, the ratio of 5β over 5α stereoisomers for all steranes is generally near the equilibrium diagnostic for abiological isomerization (average $5\beta/5\alpha = 0.65 \pm 0.26$, $n = 54$, Figs. 1D,E). By contrast, in the fossils, 5β/5α of cholestane is markedly elevated, up to 5.5 in *Dickinsonia* (Table 1, Figs. 1C,D,E), values that are generated by strictly anaerobic microbial activity (*26, 27*) such as during the decay of carcasses. Though gut flora of some mammals are known to produce 5β-stanols (precursors of 5β-steranes) (*28*), high relative abundance of these molecules in some background sediments (Fig. 1E) and macroalgae (*17*) from the White Sea contests the otherwise exciting possibility that 5β-steranes originated from *Dickinsonia*'s gut microbiota (see Supplementary Text). Notably, 5β ergostanes and stigmastanes in the *Dickinsonia* extracts are not elevated (Table 1), demonstrating that they are ultimately not derived from dickinsoniids but from the underlying microbial mat or surrounding sediment (Fig. S2). Based on these steroid homologue and isomer patterns, we compute that the sterols of living *Dickinsonia* consisted of at least 99.7% cholesteroids (Supplementary Information). Within analytical precision, it is impossible to exclude that *Dickinsonia* produced traces of ergosteroids (up to 0.23%) or stigmasteroids (up to 0.07%). Such steroids, if present, may be

derived from the organism itself, but could also represent dietary uptake or contributions from symbionts.

Biomarker signatures of *Andiva* specimens from the Zimnie Gory locality are less well differentiated from the microbial mat background signal and do not display a clear elevation of cholesteroids relative to the background (Table 1). Yet, even in these fossils, 5β/5α ratios for cholestanes are significantly higher (5β/5α = 1.02–1.31) when compared to ergostanes and stigmastanes from the fossil extract (5β/5α = 0.52–0.66) and the surrounding mat (5β/5α = 0.65–0.81; Table 1). Based on these values we can compute a conservative minimum C_{27} sterol content of 88.1% for *Andiva* (Supplementary Text).

Using the remarkable steroid patterns of the fossils, it is possible to test the position of dickinsoniids on the phylogenetic tree. Lichen-forming fungi only produce ergosteroids, and even in those that host symbiotic algae, ergosteroids remain the major sterols (*29*, *30*). *Dickinsonia* contained no, or a maximum of only 0.23% ergosteroids, conclusively refuting the lichen hypothesis (*7*). The groups of rhizarian protists that include gigantic representatives (Gromiidae, Xenophyophorea and other Foraminifera), and their retarian relatives, all produce a complex mixture of sterols, with cholesteroids comprising 10.3 to 78.2% of the mixture, ergosteroids 4.9 to 43.0% and stigmasteroids 7.2 to 60.1% (Table S4). Moreover, rhizarian protists may produce C_{30} sterols (24*n*-propylcholestane) that can form a significant (up to around 20%) proportion of their total sterol content (*31*). By contrast, in most *Dickinsonia* and *Andiva* extracts, C_{30} steroids were below detection limits. Thus, the steroid composition of dickinsoniids is markedly distinct from steroid distributions observed in Rhizaria, rendering a protozoan affinity of these fossils extremely unlikely. All animals, with rare exceptions such as some demosponges and bivalve molluscs, are characterised by exclusive production of C_{27} sterols (*32*, *33*). The closest relatives of metazoans, *Choanoflagellatea* and *Filasterea*, produce 90–100% and 84–100% of cholesterol, respectively, and contain up to 16% ergosteroids (*34*-*36*). While the sterol composition of some choanoflagellates and filastereans falls within the range observed for *Dickinsonia* and *Andiva*, they are unlikely precursor candidates since these groups are only ever represented by microscopic organisms, leaving a stem- or crown-group metazoan affinity as the only plausible phylogenetic position for *Dickinsonia* and its morphological relatives.

Molecular fossils firmly place dickinsoniids within the animal kingdom, establishing *Dickinsonia* as the oldest confirmed macroscopic animals in the fossil record (558 Ma) next to marginally younger *Kimberella* from Zimnie Gory (555 Ma) (*37*). However alien they looked, the presence of large dickinsoniid animals, reaching 1.4 m in size (*38*), reveals that the appearance of the Ediacara biota in the fossil record is not an independent experiment in large body size but indeed a prelude to the Cambrian explosion of animal life.

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Supplementary Materials:

Materials and Methods

Supplementary Text

Figs. S1 to S6

Tables S1 to S4

References (39-55)

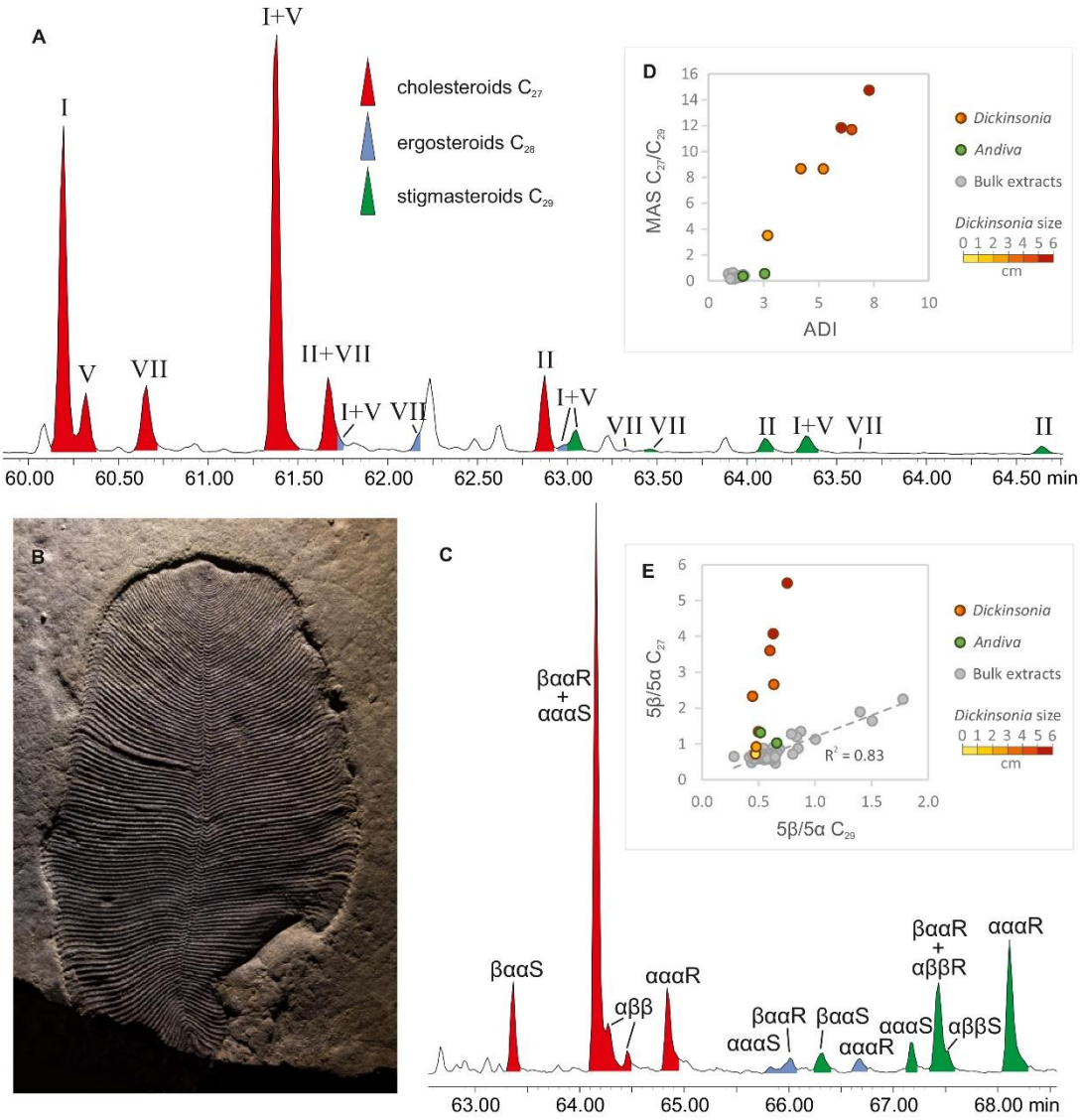

Fig. 1. Biomarkers from organically preserved *Dickinsonia*. (A) *m/z* 253 selected ion recording (SIR) chromatogram showing the distribution of monoaromatic steroids (MAS) of the solvent extract of a large *Dickinsonia* specimen (*Dickinsonia*-2; 5.5 cm width); **(B)** organically preserved *Dickinsonia* from the Lyamtsa locality (*Dickinsonia*-2); **(C)** metastable reaction monitoring (MRM) chromatogram showing the sum of $C_{27–29}$ sterane traces of *Dickinsonia*-2; αββ = 5α(H),14β(H),17β(H) (and correspondingly for ααα and βαα), S and R indicates isomerisation at position C-20; **(D)** relationship between the MAS C_{27}/MAS C_{29} ratio and the Animal Decomposition Index ADI = (C_{27} 5β/5α)/(C_{29} 5β/5α)) in *Dickinsonia* (*n* = 6), *Andiva* (*n* = 2) and bulk rock extracts from the Lyamtsa and Zimnie Gory localities (*n* = 32); only samples with detectable MAS were used in the plot; ADI is a measure of the quantity of sterols that decomposed in the anaerobic microenvironment of an animal carcass relative to normal sterol decomposition within the background sediment. ADI ≈ 1 indicates that cholesteroids and stigmasteroids underwent alteration in the same diagenetic environment consistent with the absence of animal tissue. ADI > 1 indicates contribution of animal steroids to the biomarker signal. **(E)** Relationship between the 5β/5α sterane isomer ratio for cholestane (C_{27}) and stigmastane (C_{29}) in *Dickinsonia* (*n* = 8), *Andiva* (*n* = 2) and bulk rock extracts from the Lyamtsa and Zimnie Gory localities (n = 54); 5β/5α = (βαα

20R+ααα 20S)/ααα 20R. MAS structures: I = 5β(H)10β(CH_3), II = 5α(H)10β(CH_3), V = 5β(CH_3)10β(H), VII = 5α(CH_3)10α(H).

Sample	Locality	Size (width, cm)	Saturated steranes								Monoaromatic steroids[4]			
			C_{27}%	C_{28}%	C_{29}%	C_{30}%[1]	C_{27} 5β/5α[2]	C_{28} 5β/5α[2]	C_{29} 5β/5α[2]	ADI[3]	C_{27} %	C_{28} %	C_{29} %	C_{27}/C_{29}
***Dickinsonia*-1**	Lyamtsa	6.0	48.1 *(1.3)*[5]	5.7 *(0.2)*	45.8 *(1.2)*	0.4 *(0.04)*	3.61 *(0.21)*	0.52 *(0.34)*	0.60 *(0.06)*	6.01 *(0.67)*	92.4 *(2.5)*	2.1 *(0.1)*	5.5 *(0.3)*	11.84 *(0.93)*
***Dickinsonia*-2**	Lyamtsa	5.5	63.2 *(1.5)*	4.9 *(0.2)*	31.9 *(0.8)*	0 *{0.05}*[6]	5.49 *(0.28)*	0.65 *(0.34)*	0.75 *(0.06)*	7.28 *(0.65)*	93.0 *(2.3)*	1.8 *(0.1)*	5.2 *(0.3)*	14.75 *(0.91)*
***Dickinsonia*-3**	Lyamtsa	4.5	47.9 *(1.5)*	6.1 *(0.3)*	46.0 *(1.4)*	0 *{0.03}*	4.08 *(0.29)*	0.75 *(0.34)*	0.63 *(0.04)*	6.50 *(0.64)*	92.5 *(3.2)*	2.7 *(0.2)*	4.8 *(0.3)*	11.71 *(1.41)*
***Dickinsonia*-4**	Lyamtsa	4.0	33.4 *(2.2)*	8.0 *(0.7)*	58.5 *(3.5)*	0 *{0.08}*	2.66 *(0.41)*	0.97 *(0.34)*	0.64 *(0.08)*	4.18 *(0.83)*	87.9 *(6.4)*	4.8 *(0.7)*	7.3 *(0.9)*	8.67 *(1.59)*
***Dickinsonia*-5**	Lyamtsa	4.0	44.8 *(0.9)*	7.6 *(0.4)*	47.6 *(1.0)*	0 *{0.01}*	2.33 *(0.10)*	0.81 *(0.34)*	0.45 *(0.02)*	5.21 *(0.32)*	91.3 *(4.5)*	3.6 *(0.4)*	5.1 *(0.5)*	8.66 *(1.81)*
***Dickinsonia*-6**	Lyamtsa	3.5	27.5 *(0.7)*	9.9 *(0.5)*	62.6 *(1.3)*	0 *{0.04}*	1.34 *(0.07)*	0.79 *(0.34)*	0.50 *(0.02)*	2.67 *(0.18)*	84.8 *(5.7)*	3.4 *(0.5)*	11.8 *(1.2)*	3.51 *(0.80)*
***Dickinsonia*-7**	Lyamtsa	1.0	17.2 *(1.2)*	6.9 *(1.2)*	75.9 *(3.6)*	0 *{0.18}*	0.71 *(0.10)*	0.22 *(0.34)*	0.47 *(0.05)*	1.49 *(0.26)*	-	-	-	-
***Dickinsonia*-8**	Lyamtsa	2.5	20.5 *(1.3)*	6.7 *(1.2)*	72.7 *(3.6)*	0 *{0.10}*	0.91 *(0.13)*	0.26 *(0.34)*	0.48 *(0.05)*	1.89 *(0.32)*	-	-	-	-
***Dickinsonia*-Sandstone**	Lyamtsa	-	11.6 *(0.3)*	8.9 *(0.2)*	78.9 *(1.2)*	0.6 *(0.04)*	0.64 *(0.04)*	0.85 *(0.34)*	0.52 *(0.02)*	1.23 *(0.08)*	11.9 *(1.0)*	16.8 *(1.5)*	71.3 *(4.4)*	0.17 *(0.01)*
***Dickinsonia*-Clay**	Lyamtsa	-	9.7 *(0.3)*	7.8 *(0.2)*	82.0 *(1.3)*	0.5 *(0.03)*	0.61 *(0.03)*	0.71 *(0.34)*	0.59 *(0.02)*	1.03 *(0.07)*	10.6 *(0.7)*	13.4 *(0.9)*	76.0 *(3.3)*	0.2 *(0.01)*
***Andiva*-1**	Zimnie Gory	8.0	24 *(0.4)*	10.3 *(0.3)*	64.3 *(0.9)*	1.3 *(0.1)*	1.07 *(0.04)*	0.76 *(0.34)*	0.68 *(0.02)*	1.55 *(0.07)*	21.5 *(0.8)*	17.5 *(0.7)*	61.0 *(1.9)*	0.36 *(0.01)*
***Andiva*-2**	Zimnie Gory	4.0	24.9 *(1.2)*	12.2 *(1.2)*	61.8 *(2.7)*	1.0 *(0.2)*	1.31 *(0.15)*	0.81 *(0.34)*	0.52 *(0.04)*	2.54 *(0.36)*	29.3 *(2.6)*	12.6 *(1.4)*	58.1 *(4.5)*	0.55 *(0.05)*
***Andiva* Mat**	Zimnie Gory	-	25.8 *(1.0)*	15.8 *(1.0)*	57.4 *(1.9)*	1.0 *(0.1)*	0.80 *(0.07)*	0.96 *(0.34)*	0.65 *(0.04)*	1.24 *(0.13)*	18.7 *(1.4)*	21.4 *(1.6)*	59.8 *(3.5)*	0.35 *(0.02)*
***Andiva*-Sandstone**	Zimnie Gory	-	37.5 *(1.0)*	10.5 *(0.3)*	51.6 *(1.3)*	0.5 *(0.04)*	0.65 *(0.04)*	0.91 *(0.34)*	0.61 *(0.03)*	1.08 *(0.08)*	25.6 *(1.7)*	15.7 *(1.2)*	58.7 *(3.4)*	0.43 *(0.03)*
***Andiva*-Clay**	Zimnie Gory	-	23.1 *(0.5)*	9.5 *(0.3)*	65.9 *(1.3)*	1.5 *(0.02)*	0.86 *(0.04)*	0.84 *(0.34)*	0.68 *(0.02)*	1.26 *(0.07)*	19.4 *(1.6)*	11.2 *(1.2)*	69.4 *(4.7)*	0.28 *(0.02)*

Table 1. Steroid distributions in *Dickinsonia* and *Andiva* extracts.

[1]C_{30} steranes are only represented by 24-isopropylcholestanes; [2]5β/5α = (βαα 20R+ααα 20S)/ααα 20R; [3]Animal Decomposition Index ADI = (C_{27} 5β/5α)/(C_{29} 5β/5α) (see Figure 1 caption); [4]only the I and V monoaromatic steroid isomers (see Figure 1 for nomenclature) were used for all computations as they display the least coelution with other peaks on the chromatogram; [5]numbers in parentheses are standard deviation values; [6]numbers in braces '{}' next to zero values represents the detection limit, i.e. the maximum of a given compound that may be present when not detected.

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Supplementary Materials for

Ancient steroids establish the Ediacaran fossil *Dickinsonia* as one of the earliest animals

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Materials and Methods

Sample collection

Biomarker analyses were conducted on organically preserved macrofossils *Dickinsonia* and *Andiva* (Fig. 1; Fig. S1), microbial mats surrounding the fossils, and bulk extracts of sedimentary rocks enclosing the fossils. Samples were collected during fieldwork in the Lyamtsa and Zimnie Gory localities of the Ediacara Biota in the White Sea Region (Russia) in 2015–2017 specifically for biomarker analysis. *Dickinsonia* (8 specimens) come from a surface in the lower part of the Lyamtsa Beds (Ust-Pinega Formation, Redkino Regional Stage), exposed to the south of Lyamtsa Village. The fossils were underlain by clay with mm-thick lenses of siltstone and sandstone, and buried under 30 cm-thick sandstone layer, composed of thinner (5-10 cm) beds with hummocky cross-stratification, separated by microbially induced wrinkle structures. *Andiva* (2 specimens) were collected from a surface in the lower part of the Erga Beds (Mezen Formation, Kotlin Regional Stage). The fossils were also underlain by clay with mm-thick lenses of siltstone and sandstone and covered with 40-60 cm thick lenses of massive sandstone with soft sediment deformations and thick (up to 5 cm thick) load cracks at the bottom. Samples used for determining the background signal (n=54) were collected from Lyamtsa and Verkhovka Beds (Ust-Pinega Formation, Redkino Regional Stage) of the Lyamtsa locality and from Vaysitsa, Zimnie Gory (Ust-Pinega Formation, Redkino Regional Stage) and Erga Beds (Mezen Formation, Kotlin Regional Stage) of the Zimnie Gory locality. All samples were collected avoiding weathered zones and cracks and were immediately wrapped in pre-combusted aluminium foil (300°C, 9h) and packed in calico cotton bags under strict avoidance of contamination (*17*).

Sterol signatures of rhizarian protists were determined on natural specimens. *Sorites* sp. and *Amphistegina lobifera* were collected attached to pebbles by snorkelling in shallow water 1-3 m in Eilat, Israel and subsequently kept under controlled laboratory conditions for four months until they were handpicked again and cleaned with a brush prior to laboratory analyses. The two Foraminifera *Shepheardella* sp. (collected at the Gulf of Eilat) and *Quinqueloculina* sp. (collected at the Mediterranean coast of Tel Aviv, Israel) were extracted shortly after collection in the field after separation and cleaning with tweezers and brushes. Brightly orange *Shepheardella* sp. allogromid Foraminifera were picked from rocks and sea-grass. Collodarian radiolarians were handpicked from the contents of a plankton net collected from surface waters in the Villefranche sur Mer bay (France). *Gromia* sp. were collected by Scuba Divers from the bottom sediments at the McMurdo Station jetty in Antarctica and pickled in ethanol. A bulk *Acantharea* sample was collected using a plankton tow net during a bloom event of *Acantharea* sp. in the shallow Twofold Bay near Eden, SE Australia that is open to the Tasman Sea. After collection, the sample was washed with freshwater and pickled in 100% ethanol, then kept frozen until analysis at the Australian National University.

Fossils characteristics

The thickness of organic matter of *Dickinsonia* was estimated based on a thin section made through one of the fossil specimens. The size of fossil specimens was determined based on their width as most of the analysed specimens were not complete and lacked either of their

ends. These size measurements are estimates as all dickinsoniids were deformed before or during burial.

Sample preparation and extraction

Organic matter of the fossils was separated from the sandstone surface using 5% hydrochloric acid (several drops on the surface of a fossil) and 30% hydrofluoric acid (several drops on the surface of a fossil, followed by drying, repeated two times). Fossils were then placed into a glass beaker filled with water to remove acid, allowing most of organic matter to detach from remaining inorganic components of the fossils. Any remaining organic matter attached to the rock was separated with a scalpel. Combined organic matter was neutralized in deionized (Millipore Elix 3UV, France) water under ultrasonic agitation and subsequently centrifuged (3 times) to remove remaining acids. Hydrocarbons were extracted from the organic matter via ultrasonic agitation in solvents (methanol for 30 min, dichloromethane (DCM) for 15 min (x 2), DCM : *n*-hexane 1 : 1 for 15 min). All solvents were 99.9% grade (UltimAR®; Mallinckrodt Chemicals, St. Louis, MO, USA). Glassware was cleaned by combustion at 300°C for 9 h.

Bulk sedimentary samples (represented by clay, heterolithic interlamination and sandstones) were analysed using the so-called Exterior-Interior protocol described in detail elsewhere (*17, 39, 40*). Briefly, exterior ('E') 3–4 mm of rock samples were removed using a micro drill (Dremel® 400 Series Digital, Mexico) with a solvent cleaned saw nozzle. Analysis of the last saw-blade solvent rinse confirmed that it was free of detectable contaminants. The exterior and the interior portions of each sample were ground to powder (>240 mesh) using a steel puck mill (Rocklabs Ltd., Onehunga, New Zealand). The mill was cleaned using methanol and dichloromethane, and by grinding combusted (600°C, 9 h) quartz sand.

Bitumen was extracted from rock powder using an Accelerated Solvent Extractor (ASE 200, Dionex, USA) with DCM : methanol (9:1), reduced to 100 µL under a stream of nitrogen gas, and then fractionated into saturated, aromatic and polar fractions using micro-column chromatography over annealed (300°C; 12 h) and dry packed silica gel (Silica Gel 60; 230–600 mesh; EM Science). Saturated hydrocarbons were eluted with 1 dead volume (DV) of *n*-hexane, aromatic hydrocarbons with 4.5 DV of *n*-hexane : DCM (1 : 1) and the polar fraction with 3 ml DCM : methanol (1 : 1). Extracts from fossils with low hydrocarbon content were only fractionated into saturated+aromatic and polar fractions to reduce the probability of contamination and loss of analytes. An internal standard, 18-MEAME (18-methyleicosanoic acid methylester; Chiron Laboratories AS), was added to the saturated and aromatic fractions, while D4 (d_4-C_{29}-ααα**R**-ethylcholestane; Chiron Laboratories AS) was added to saturated and saturated+aromatic hydrocarbon fractions with low biomarker content. The samples were analysed and quantified by GC–MS.

Biomass of rhizarian protists (Extended Data Table 4) was extracted thrice using a modified Bligh and Dyer method (DCM:MeOH:H_2O=1:2:0.4, then 2:2:0.4, then 2:2:1.4) or by ultrasonic agitation in DCM : MeOH (9 : 1). *Gromia* specimens were stored in ethanol after sampling and the ethanol was analysed in the same manner as the other extracts. The total lipid extracts were separated into hydrocarbons (F1), esters and ketones (F2), alcohols (F3) and fatty acids (F4) by small-scale open column chromatography over aminopropyl-substituted silica gel (Chromabond Sorbent NH2, Macherey-Nagel, Germany; 500 mg in a 6 mL glass SPE column), where F1 was eluted with 4 mL *n*-hexane, F2 with 6 mL n-hexane:DCM (3:1), F3

with 7 mL DCM:acetone (9:1) and F4 with 8 mL DCM containing 2% formic acid. F3 was hydrogenated by bubbling a gentle stream of hydrogen gas into a 4 mL vial filled with n-hexane, F3 lipids and platinum (IV) oxide (ca. 7 mg) under stirring for >4h. Converted steranes were purified by eluting with an excess of *n*-hexane over a small chromatographic column filled with activated silica gel. Blank hydrogenations were run before and after samples to assess contamination. *Acantharea* sp. lipids were extracted with a similar modified Bligh and Dyer method, and saponified with 5 % KOH in MeOH : H_2O (8 : 2) and neutral lipids were recovered by addition of H_2O and the same amount of Hex : DCM (4:1), pyrolysed at 320 °C for 21 h in an evacuated glass tube and hydrogenated with PtO_2 overnight.

Gas chromatography–mass spectrometry (GC-MS)

GC-MS analyses of Ediacaran and Acantharea extracts were carried out on an Agilent 6890 gas chromatograph coupled to a Micromass Autospec Premier double sector mass spectrometer (Waters Corporation, Milford, MA, USA) at the Australian National University. The GC was equipped with a 60 m DB-5 MS capillary column (0.25 mm i.d., 0.25 µm film thickness; Agilent JW Scientific, Agilent Technologies, Santa Clara, CA, USA), and helium was used as the carrier gas at a constant flow of 1 mL min^{-1}. Samples were injected in splitless mode into a Gerstel PTV injector at 60°C (held for 0.1 min) and heated at 260°C min^{-1} to 300°C. For full-scan, selected ion recording (SIR) and metastable reaction monitoring (MRM) analyses, the GC oven was programmed from 60°C (held for 4 min) to 315°C at 4°C min^{-1}, with total run time of 100 min. All samples were injected in *n*-hexane to avoid deterioration of chromatographic signals by $FeCl_2$ build-up in the MS ion source through use of halogenated solvents (*41*). *n*-Alkanes were quantified in full-scan mode in *m/z* 85 trace, and ααα20R-stigmastane – in *m/z* 217 trace, relative to the 18-MEAME internal standard (*m/z* 340). Steranes and hopanes were quantified in MRM mode in $M^+ \rightarrow 217$ and $M^+ \rightarrow 191$ transitions respectively relative to ααα 20R-stigmastane. Monoaromatic steroids were quantified in SIR mode using the *m/z* 253 base ion. Peak areas are uncorrected for differences in GC-MS response.

Hydrogenated rhizarian steroids were analysed on a Thermo Quantum XLS Ultra triple quadrupole MS coupled to a Thermo Trace GC at the University of Bremen. The GC was fitted with a VF-1 ms (40m; 0.15 µm; 0.15 mm), DB-XLB (60 m; 0.25 mm; 0.25 µm) or DB-35 ms (60 m; 0.25 mm; 0.25 µm) capillary column and operated under a constant flow (1.3 mL/min.) of helium (99.999% pure, Westfalen AG). Samples were injected in splitless mode using a Thermo PTV injector, while the oven was ramped from 60ºC (5 mins. isothermal) to 325ºC at 4º/min. and held at final temperature for 25 mins. Electron impact ionization was achieved at 70 eV and 250ºC with an emission current of 50 mA. The MS was operated in 0.7 Da resolution (Q1 and Q3) with a cycle time of 0.5 sec, while Q2 was operated with Argon (99.999% pure; Westfalen AG) at a pressure of 1.1 mtorr and varying collision voltages. Analytes were identified in comparison to the NSO-1 oil standard and quantified on characteristic parent-to-daughter ion mass transitions (*m/z* 372, 386, 400 and 414 fragmenting to 217) without correcting for differences in response. Ediacaran biomarker peaks were identified based on relative retention time in comparison with AGSO industrial standard (a standard mixture of oils of different ages) and literature data. Identification of βαα sterane isomers was additionally confirmed by mass spectra obtained in full-scan mode.

Error quantification

As repeat injections of fossil extracts were not possible due to the limited abundance of valuable sample material, standard deviations for the concentration of each biomarker were externally determined using the AGSO industrial standard. The main sources of quantification error in extracts are low signal to noise ratios of molecular peaks in mass chromatograms. We explored the relationships between signal/noise ratio and coefficient of variation for our specific GC-MS system for each quantified compound using five repeat injections of the AGSO Standard. MRM signal areas of steranes, hopanes, cheilanthanes and the D4 internal standard were integrated for each repeat run and plotted against the coefficient of variation C_v for the five repeats (Fig. S3; C_v was computed for the ratio of compound signal relative to the highest biomarker peak, C_{30} 17α(H)21β(H) hopane). The plot yields a relationship described by the formula $C_v = 186.18x^{-0.31}$ ($R^2 = 0.82$), where x is the integrated peak area. The standard deviation for each GC-MS signal in sample extracts used in this study was calculated based on this relationship (STD = C_v*signal area). For biomarker ratios, error propagation followed standard root mean square error propagation formulae. As an estimate of the maximum content of compounds that were below detection limits, the smallest visible peak in corresponding chromatograms was integrated.

As βαα 20R-ergostane (5β) coelutes with two other compounds, ααα 20S-ergostane and an unknown sterane (tentatively identified as a βαα 20R-27-nor-sterane), its integration error should be expected to be much higher than the error for peaks that are not coeluting and are mainly controlled by signal/noise ratio. Therefore, we estimated an error by comparing measured and computed values for 5β 20R-ergostane. In bulk extracts, 5β/5α ratios should theoretically be identical for all three sterane pseudohomologues, i.e. for cholestanes, ergostanes and stigmastanes (*26*). We thus measured the 5β/5α ratios of cholestanes and stigmastanes in bulk sediment extracts (Fig. 1E) and used the average of 5β/5α of cholestanes and stigmastanes in each individual sample to predict a theoretical 5β/5α value for ergostanes. The average deviation of calculated and measured values across 54 samples was used as the standard deviation for this ratio.

Laboratory system blanks

A comprehensive, accumulatory system blank was performed covering all analytical steps from rock surface removal, extraction, fractionation to instrumental analysis. For this purpose, combusted (600°C, 9 h) sand was used to monitor background contamination during grinding, extraction, and fractionation processes.

Supplementary Text

Biomarker composition of dickinsoniids

5β/5α sterane isomers ratio

The elevated 5β/5α ratio of cholestane (up to 5.5) detected in all dickinsoniid fossils (Table 1, Figs. 1C,D,E) is rarely observed in nature and the geological record. Yet more unusual, in the dickinsonniids only cholestane 5β/5α is elevated while the corresponding ergostane and stigmastane ratios show the typical sedimentary background value 5β/5α ≈ 0.6. Such a discrepancy between sterane pseudohomologues has, to our knowledge, never been observed before in the fossil record. Under usual sedimentary conditions, the 5β isomer is generated by hydrogenation of Δ^5 sterenes (sterenes with a double bond in position 5 of the ring system), while 5α is formed from Δ^4 sterenes. The biological configuration of most biological sterols is Δ^5. However, due to double bond isomerisation between the Δ^5 and Δ^4 positions, the equilibrium ratio between the 5β and 5α sterane isomers in an abiological thermodynamically driven system is $\Delta^5 \rightarrow 5\beta : \Delta^4 \rightarrow 5\alpha = 39:61$, resulting in 5β/5α = 0.64 (*26*). This proportion is characteristic of bulk rock extracts from the White Sea Area (average 5β/5α = 0.64 ± 0.26, n = 54), but is much lower than 5β/5α cholestane ratios in all dickinsoniid fossils. An origin of such elevated 5β/5α sterane ratios as observed in *Dickinsonia* can be explained by microbial or dietary hydrogenation of unsaturated sterols to 5β-stanols, common under strictly anaerobic conditions, and their further dehydration to Δ^3 5β-sterenes and subsequent reduction to 5β-steranes (*26, 27, 42*). These processes, when occurring within sediment, affect all steroid pseudohomologs equally, regardless of side chain methylation (*26*). In order to create the elevated 5β/5α cholestane ratio observed in the dickinsoniid extracts, only C_{27} sterols could have been subject to this mode of decay in a strongly anaerobic microenvironment. Sterols from the surroundings of the fossils were degraded under different taphonomic conditions, allowing us to discern the signal of fossils and background.

In principle, the elevated 5β/5α cholestane ratios and the strong aromatization of cholesteroids (Table 1) in dickinsoniids may arise from the presence of an unusual C_{27} steroid, e.g. possessing multiple unsaturations. Additionally, the gut microbiota of some mammals, including humans, transforms cholesterol to coprostanol (5β-cholestanol) (*28*), resulting in the formation of 5β-steranes during diagenesis. Thus, it is tempting to speculate that dickinsoniids possessed a gut or other digestive systems, and also microbiota responsible for this process. However, elevated 5β/5α ratios were also detected for C_{27} to C_{29} steranes in some sediment extracts (5β/5α = up to 2.3; Fig. 1E) and in Ediacaran macroalgae from the White Sea with 5β/5α as high as 3.7 for cholestane, ergostane and stigmastane (Fig. S4) (*17*). The data show that elevated 5β/5α ratios form in sedimentary environments during decomposition of organic matter, and that these processes are not restricted to Ediacaran macrofossils.

The elevated 5β/5α cholestane ratio in dickinsoniid fossil extracts is consistent with an origin from decomposing animal tissues. The occurrence of 5β-stanols has been recorded even in freshly collected marine animals on initial stages of decomposition (*43, 44*). In such cases, formation of 5β-stanols can be connected with microbial transformation of animal tissue, independent of the structure of the precursor sterol. For instance, in decaying sponges, unusual sponge sterols were also found to be affected by this process, resulting in formation of 5β-dihydro stanols (*45*). Anaerobic degradation of lipids via hydrogenation also dominates in human remains, which is well studied in forensic taphonomy (*46, 47*).

Determination of exact sterol composition of Dickinsonia

Dickinsoniids inhabited a sea floor covered by microbial mats (Fig. 1B, Figs. S1,S2). Such microbial mats presumably developed over weeks, months or even years, accumulating senescent biomass of mat inhabitants and plankton. After burial by mud or sand, the organic matter of mats, plankton and dickinsoniids fused (Figs. S2A,B,C), making it impossible to physically separate the biomass. Thus, to determine the molecular composition of dickinsoniids, the molecular signal from the underlying mat needs to be separately determined and subtracted.

The determination of the background signal coming from the mat is a technical challenge. Measurement of the molecular signal of the mat directly surrounding the fossils (e.g. Fig. S1A) yielded extracts with extremely low signal/noise ratios (Table 1) and thus intolerably high propagated errors, or did not yield any detectable biomarkers. Moreover, it was unclear whether decaying and liquefied biomass from dickinsoniids would spill along the bedding plane, creating a halo around the carcass and affecting the taphonomy of molecules that do not belong to the organism. Furthermore, dickinsoniid fossils may contain dietary organic matter ('gut content', if dickinsoniids had a gut (*48*)), and this organic matter would be transformed in similar microenvironments as indigenous dickinsoniid tissue, complicating the signal further.

Variations in steroid composition of the extracts of different *Dickinsonia* specimens can be described by a two-component mixing system curve as a result of mixing between the steroids of the background signal and steroids directly derived from *Dickinsonia* (Fig. S2D). The mixing scenario is generally described by $x = n*x_1 + (1-n)*x_2$ where x is the concentration of a compound in the mixture, and x_1 and x_2 are the concentrations of that compound in the two components. In Figure S2D, the steroid composition for the background was taken as the average of sediment directly under- and overlying *Dickinsonia* (Table 1), and the concentrations of steranes and MAS in *Dickinsonia* were adjusted to best describe the data. The resulting relationship between sterane C_{27} (%) and MAS C_{27} (%) allows to estimate that *Dickinsonia* largely or exclusively produced C_{27} sterol (~95–100%) and that aromatisation of sterols during the decay of *Dickinsonia* was more pronounced than in the background (Fig. S2D). The extrapolation, however, is not precise enough to discern whether living *Dickinsonia* contained small quantities of indigenous C_{28} and C_{29} sterols.

We were able to obtain a more precise sterol composition of *Dickinsonia* by quantifying bacterial hopanes in several *Dickinsonia* specimens. Hopanes are largely derived from aerobic bacteria; they are extremely uncommon in anaerobes (*49*). As *Dickinsonia* was degraded under largely anaerobic conditions (see above), the hopanes in the fossil extracts must be largely derived from the underlying mat or the surrounding sediment. Thus, the hopane content in individual *Dickinsonia* extracts is a measure for the magnitude of the background signal. To subtract the background and to estimate the relative proportion of cholesteroids, ergosteroids and stigmasteroids of living *Dickinsonia* we used the three largest specimens with the best signal/noise ratios (Table 1). First we quantified the variance of the three eukaryotic monoaromatic steroids relative to hopanes between the *Dickinsonia* extracts (Table S1). The steroid/hopane ratio of steroids that are solely derived from the background should stay constant, while the ratio for steroids produced by *Dickinsonia* as well as the background should vary with a varying *Dickinsonia*/background biomass ratio. To determine this mathematically, for each of the MAS/H ratios we used a standard MS Office Excel variance VAR.S formula

VAR.S = $\sum(x - \bar{x})^2/(n-1)$, where x is a MAS/H value for each MAS homolog in an extract, $\bar{x}$ is a mean value between the extracts and n is the number of samples (n = 3).

The ratios of ergosteroids and stigmasteroids over hopanes remained nearly constant across the three fossil extracts with very small variances ($0.076*10^{-4}$ for ergosteroids and $0.024*10^{-4}$ for stigmasteroids; Table S1). By contrast, the content of cholesteroids relative to hopanes changed with a three order of magnitude higher variance ($33*10^{-4}$). These data indicate that the bulk of cholesteroids must come from the dickinsoniids while almost all of the ergosteroids and stigmasteroids, together with the bacterial hopanes, represent a constant background signal, presumably coming from the underlying microbial mat containing biomass of bacteria and algae. Based on the computed variances, the proportion of each steroid variability can be estimated in % relative to the total variability (sum of all three steroid/hopane ratios). Such quantification of *Dickinsonia* steroid composition is based on the assumption that the background has no variability in steroid homolog proportions or relative hopane abundances. This is a conservative assumption because if the steroid/hopane ratios of the background were not constant, then ergosteroid and stigmasteroid variability would increase and their estimated content in dickinsoniids would go up. Based on these assumptions, we compute a minimum cholesteroid content of *Dickinsonia* of 99.7%, while the maximum ergosteroid content is 0.23% and stigmateroids 0.07% (Table S1).

Similar computations can be made based on variability of 5β/5α sterane ratios in *Dickinsonia* extracts. Unfortunately, an assumption that the background signal is invariable, necessary for this computation, does not fully apply to 5β/5α sterane background ratios (an average 5β/5α value between all background signals is 0.65, ranging however from 0.28 to 1.78, n = 54; Fig. 1C). In our computations, this variability in the background signal, which is likely due to natural variations in diagenesis (see above), would artificially elevate the ergosteroid and stigmasteroid proportion of *Dickinsonia*. However, even based on this imperfect data, the computation yields a conservative minimum C_{27} steroid content for *Dickinsonia* of 98.4%, while the maximum proportions of ergosteroids and stigmasteroids are 1.1% and 0.54% respectively (Table S1).

Estimation of the sterol composition of Andiva

Andiva specimens for this study were exclusively recovered from the Zimnie Gory locality. At Zimnie Gory, the proportion of sedimentary background signal in fossil extracts was far higher than at the Lyamtsa locality, where *Dickinsonia* was recovered. Thus, for *Andiva* it is more difficult to separate the dickinsoniid signal from the background. Moreover, the methodology employed for *Dickinsonia*, i.e. computation of the variance of MAS/hopane ratios, was not possible for *Andiva* because the background MAS signal is too high. However, a rough estimate of the sterol composition of *Andiva* was possible based on 5β/5α sterane ratios the same way as for *Dickinsonia*, but in this case using variability in 5β/5α ratios for each sterane between the analysed *Andiva* extracts and an extract of surrounding microbial mat. With the assumptions discussed above, the most conservative estimate for the minimum C_{27} steroid content of *Andiva* is 88.1%, while the maximum proportions of ergosteroids and stigmasteroids are 1.2% and 10.7% respectively (Table S2).

Other biomarkers

Hopanes

All extracts exhibit typical immature hopane distributions with low Ts/(Ts+Tm) and S/(S+R) ratios, high βα/(βα+αβ) ratios and high relative abundances of C_{27}, C_{29} and C_{30} homologs relative to the extended C_{31} to C_{35} homohopanes (Table S3, Figs. S5C,D). An unusual elevated proportion of C_{27}–C_{30} hopanes in *Dickinsonia* and *Andiva* extracts, relative to bulk extracts from the Zimnie Gory and Lyamtsa localities, might indicate relatively large proportions of cyanobacteria among hopanoid-producing bacteria in the underlying microbial mats when compared to bulk organic matter. However, the unusual hopane pattern may also be the result of differences in the diagenesis of hopanoids between organic films and bulk samples (*17*).

Methylhopanes

The 2-methylhopane index (2-MHI) measures the abundance of C_{31} 2-methylhopane relative to C_{30} hopane (Table S3). 2-MHI is low and similar in all analysed fossils where 2-methylhopanes were detected (2-MHI = 0.8–1.8%). Elevated 2-MHI have been interpreted as an indicator for significant cyanobacterial contribution to sedimentary organic matter (*50*). However, only a minority of cyanobacteria have the ability to produce 2-methylhopanoids (*51*), and their content in cyanobacteria significantly depends on growth conditions (*52*). Apart from cyanobacteria, 2-methylhopanoids can also be produced by other oxygenic and anaerobic bacteria including alphaproteobacteria and an acidobacterium (*51*). Thus, in light of the unspecific taxonomic distribution of 2-methylhopanoids, and environmental control on their biosynthesis (*51, 53*), the 2-MHI values in the fossils do not yield any further insights about biological origins. 3β-methylhopanes were detected in small amounts in the samples (Table S3) and might reflect a minor contribution of methanotrophic bacteria to the organic matter of the samples (the 3-methylhopane index values 3-MHI = 1.1–2.6%) (*54*).

n-Alkanes and methylalkanes

Bulk extracts of background sediment from the Zimnie Gory and Lyamtsa localities demonstrate odd-over-even predominance among *n*-alkanes at all chain lengths and, unusually for the Precambrian, a relatively large proportion of long-chain *n*-alkanes with odd-over-even predominance (Figs. S5A,B). Such a distribution might indicate that a large proportion of *n*-alkanes is derived from cyanobacteria or green algae (*17*). All *Andiva* and the leanest *Dickinsonia* extracts do not provide sufficient signal for *n*-alkane analysis. The richest *Dickinsonia* extracts demonstrate the same *n*-alkane pattern as the background, however the fossil extracts additionally contain large proportions of short-chain (up to C_{19}) *n*-alkanes with no odd-over-even predominance (Fig. S5A). These *n*-alkanes are presumably directly derived from *Dickinsonia* or from degrading bacteria. However, such compounds are widely distributed in nature and do not provide any further phylogenetic information. All samples demonstrate only low amounts of methylalkanes, which likely represent diagenetic products of *n*-alkanes formed through clay mediated catalysis (*55*).

Other saturated and aromatic hydrocarbons

The majority of hydrocarbons extracted from the analysed samples are represented by *n*-alkanes, steranes and hopanes, with minor amounts of cheilanthanes, which are ubiquitous molecules of unknown origin. Aromatic compounds, apart from MAS and triaromatic steroids (TAS), include polycyclic aromatic hydrocarbons represented by typical parent and alkylated phenanthrenes and naphthalenes found in most bitumens and oils. TAS follow the patterns of distribution of MAS, demonstrating slightly lower relative content of cholesteroids (e.g. in the richest *Dickinsonia*, cholesteroid : ergosteroid : stigmasteroid abundances = 86% : 5% : 9%).

Syngeneity of biomarkers

GC-MS MRM, SIR and full-scan analyses of the comprehensive accumulatory laboratory system blank confirmed that the detected hydrocarbons were not introduced by laboratory processes. Monitoring of the blank yielded no *n*-alkanes, hopanes or steranes, even when measured using the most sensitive GC-MS MRM methods (Fig. S6). The only contaminants detected in the blank are trace amounts of phthalates—plasticizers present in small amounts in solvents used for extraction of biomarkers.

The results of Exterior/Interior (E/I) experiment, as well as a detailed discussion of biomarkers syngeneity of the samples from the White Sea that were collected, transported and stored in the same way as samples used in this study are presented elsewhere (*17*). The results show nearly identical concentrations of all biomarkers in exterior and interior rock portions with E/I = 1.09 to 1.53 for *n*-alkanes and E/I = 0.93 to 1.05 for hopanes and steranes, which indicates that the precombusted aluminium foil and clean calico bags prevented contamination of samples during transportation and storage, and confirm that all detected compounds are indigenous (*17*).

Dickinsoniid extracts demonstrate the same exceptionally low thermal maturity as *Beltanelliformis* and organically preserved macroalgae from the Lyamtsa locality (*17*). The high βα/(βα + αβ) and low Ts/(Ts +Tm) hopane isomer ratios, virtual absence of diasteranes and αββ sterane isomers, and presence of βαα steranes (Fig. 1C; Table S3), which are only found in the most immature sediments, demonstrate that the hydrocarbons are significantly below the so-called oil-generative window, i.e. the kerogen never thermally generated and expelled liquid hydrocarbons (*54*). Such a low thermal maturity is never observed in contaminant petroleum products and migrated oils, which by their very nature must have a maturity within the oil generative window. Thus, contamination of the samples by petroleum products or by migrated hydrocarbons did evidently not occur.

Supplementary Figures

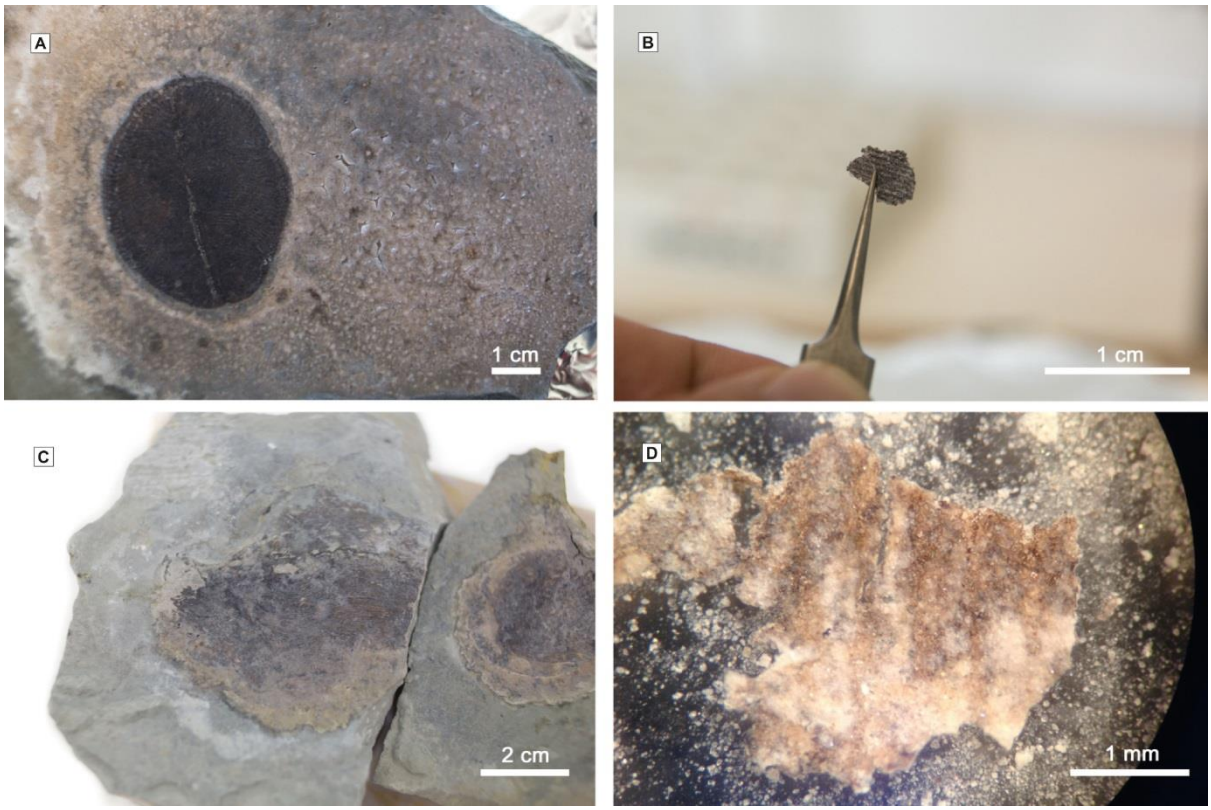

Fig. S1. Separation of organic matter from dickinsoniids. (A) Organically preserved *Dickinsonia* (specimen 4) after treatment with hydrochloric and hydrofluoric acids. Note the thin film of organic matter covering the surface with microbial mat impression surrounding the fossil; **(B)** a piece of organic matter of *Dickinsonia*-3 separated from the specimen with tweezers; **(C)** organically preserved *Andiva* (specimen *Andiva*-1) after treatment with hydrochloric and hydrofluoric acids, the organic matter is more visible as it starts separating from the rock surface where the acids were applied; **(D)** organic matter of *Andiva*-1 suspended in water after separation from the specimen showing preservation of *Andiva*'s fine structure.

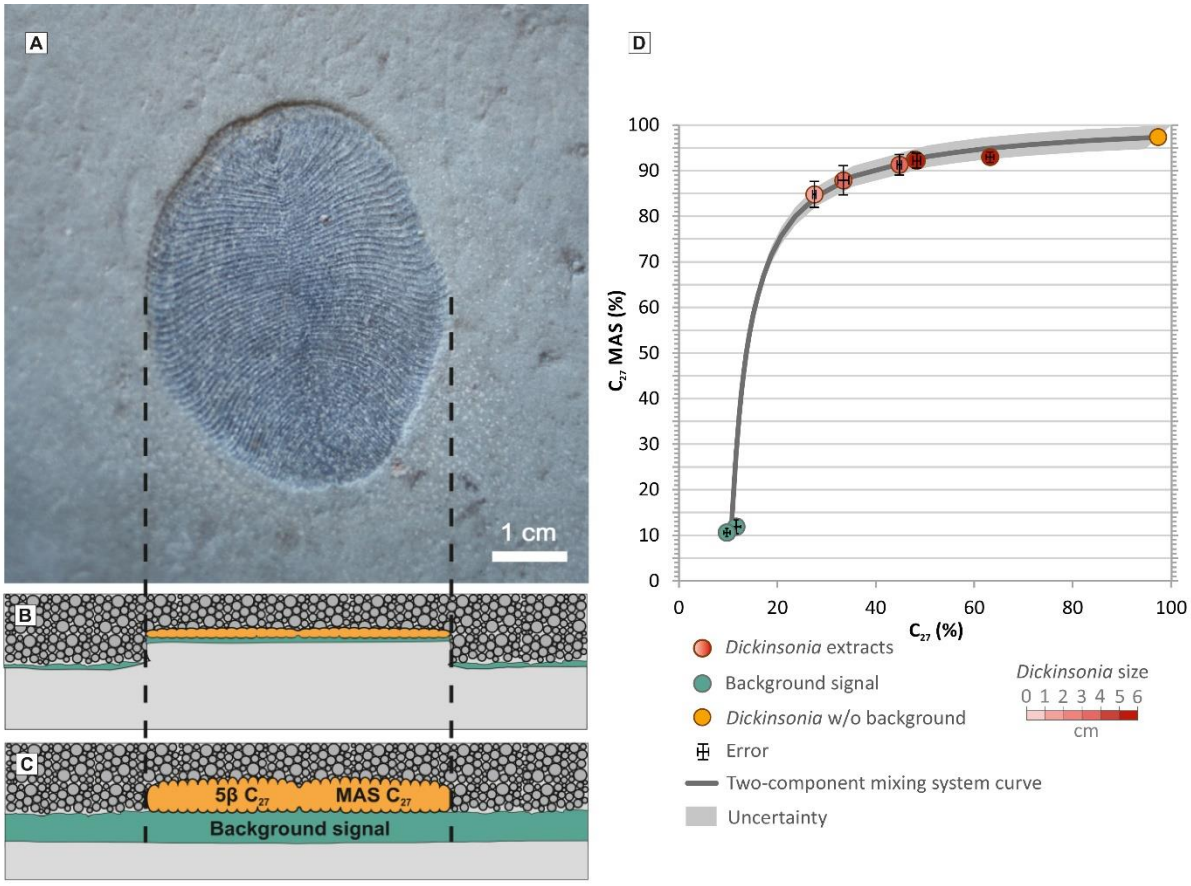

Fig. S2. Explanation of the origin of different biomarker signals of dickinsoniid extracts. **(A)** Organically preserved *Dickinsonia* fossil (specimen 4) and surrounding impression of microbial mat; **(B)** schematic cross-section of the fossil, showing the position of organic matter of the microbial mat (green) and *Dickinsonia* (orange); **(C)** cross-section reconstructing the position of microbial mat and *Dickinsonia* immediately after burial beneath sand, highlighting the origin of the background signal in dickinsoniid extracts and explaining its variable influence depending on the size of a *Dickinsonia* specimen; **(D)** plot showing relationship between relative proportion of cholesteroids among steranes (C_{27} (%)) and monoaromatic steroids (C_{27} MAS %); the dark-grey curve represents a numerically solved two component mixing scenario, and the light-grey area represent uncertainty of this curve (Supplementary Text).

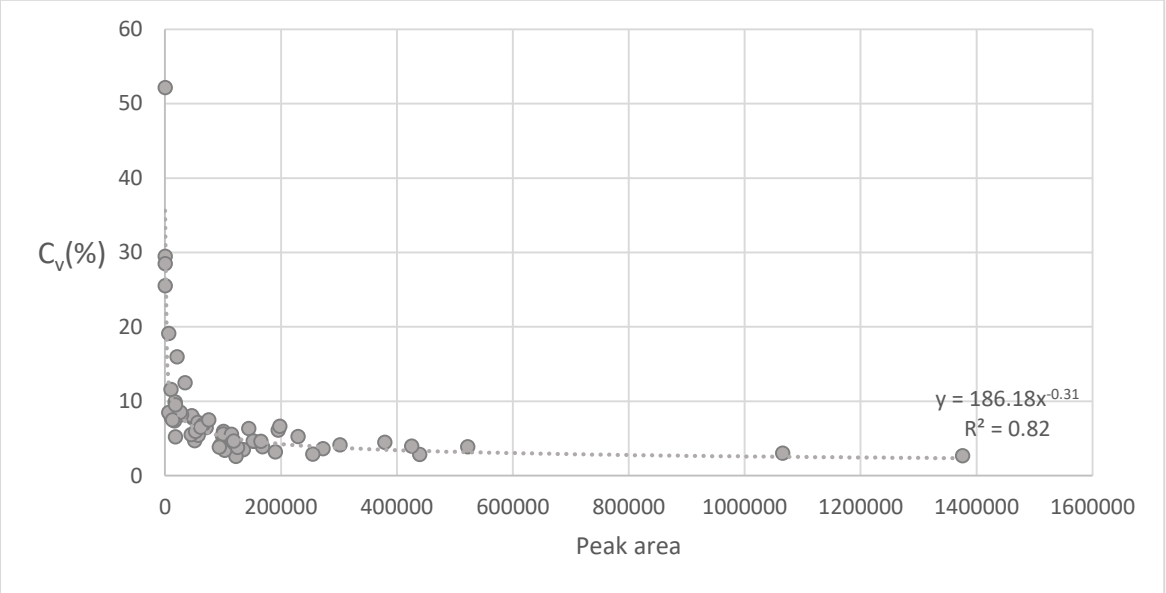

Fig. S3. Relationship between measured biomarker peak area and coefficient of variation (C_v). Each data point represents a different sterane or hopane in the AGSO-II industrial standard oil mixture, and the peak area is the dimensionless Metastable Reaction Monitoring (MRM) chromatographic signal area of the average of five repeat injections. Cv was computed for the ratio of compound signal area normalized to C_{30} 17α(H)21β(H) hopane, which represents the highest signal in the MRM chromatograms. In the formula describing the relationship between measured biomarker peak area and Cv ($y = 186.18x^{-0.31}$), y = Cv, x = peak area.

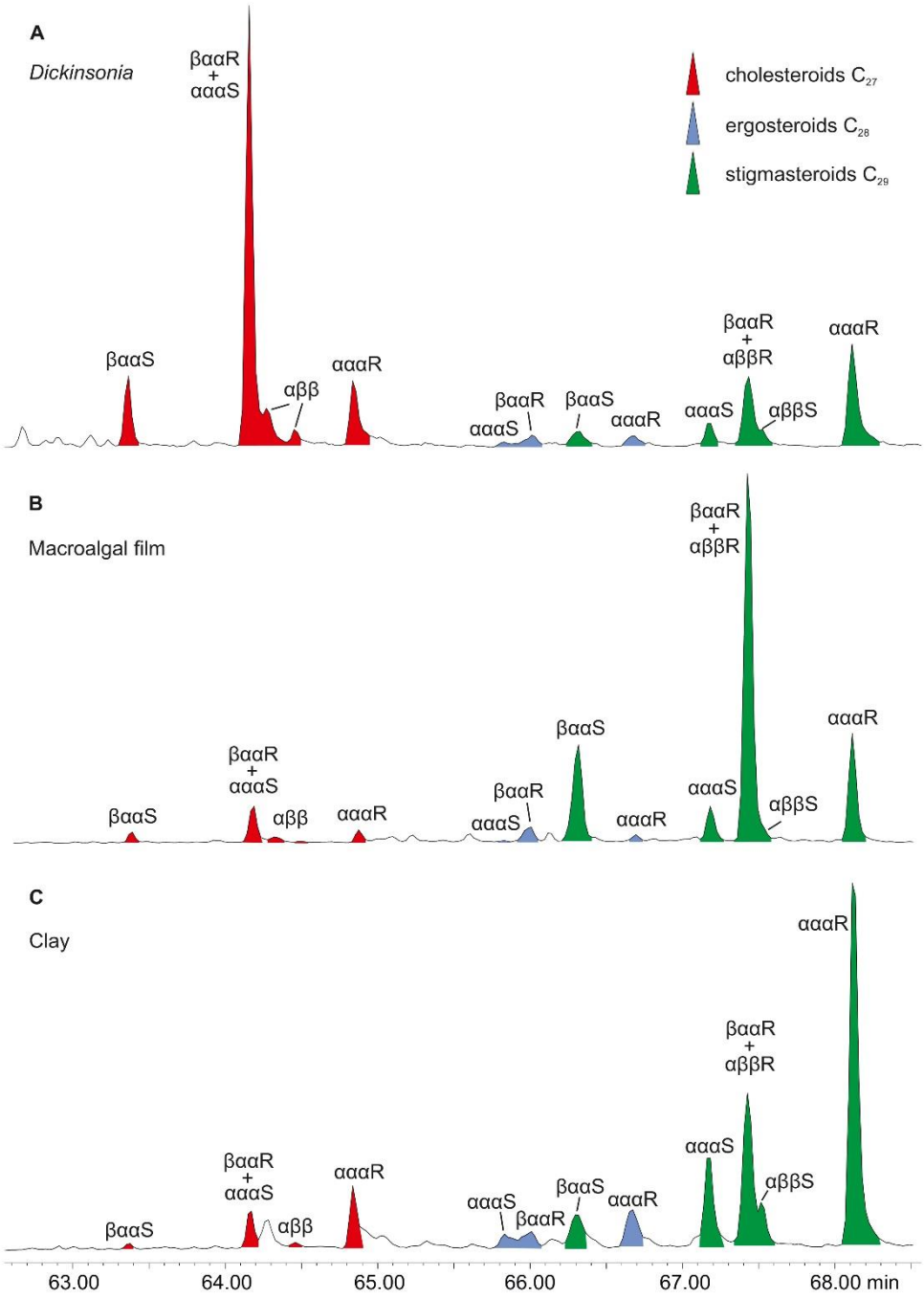

Fig. S4. Comparison of the sterane composition of *Dickinsonia*-2 macroalgal film and background sediments from the Lyamtsa locality. (A) *Dickinsonia*-2 displays elevated 5β/5α = 5.5 for cholestane only, while it is ~0.7 for ergostane and stigmastane; **(B)** the macroalgal film extract demonstrates elevated 5β/5α ratios for all three steranes with 5β/5α = 3.7; **(C)** clay immediately underlying the level with *Dickinsonia* demonstrates typical background 5β/5α ratio values of 0.64 for all three steranes. The elevated 5β/5α ratios in the alga demonstrate that 5β steranes can form in sedimentary environments during decomposition of organic matter and that the process is not restricted to microbial activity within the digestive system of animals (*28*) and is not caused by a specific nature of cholesteroid in *Dickinsonia* and *Andiva*.

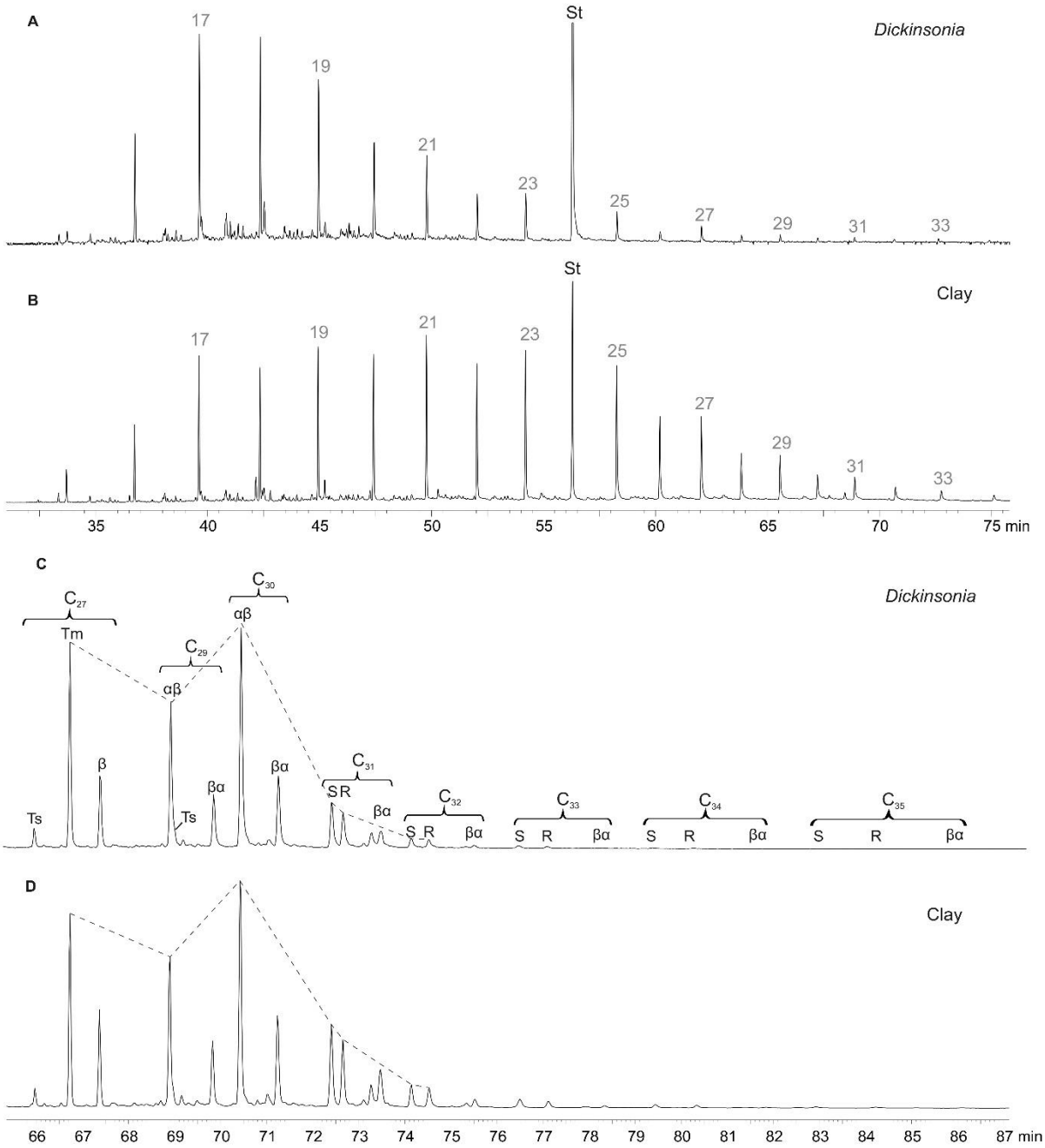

Fig. S5. Distribution of *n*-alkanes and hopanes in *Dickinsonia*-2 and the underlying clay extracts. (A) Full-scan chromatogram, *m/z* 85 trace of *Dickinsonia*-2 extract showing the distribution of *n*-alkanes; **(B)** full-scan chromatogram, *m/z* 85 trace of clay extract underneath *Dickinsonia* showing the distribution of *n*-alkanes; **(C)** Metastable Reaction Monitoring (MRM) chromatogram showing the sum of $C_{27–35}$ hopane traces of *Dickinsonia-2* extract; **(D)** MRM chromatogram showing the sum of $C_{27–35}$ hopane traces of clay extract. Numbers on chromatographic signals in (A) and (B) indicate the carbon number of *n*-alkanes, St = internal standard 18-MEAME. The dotted line in (C) and (D) highlights the difference in hopane ($C_{27–30}$) and homohopane ($C_{31–35}$) relative abundances between *Dickinsonia* and clay extracts. Hopane structures: αβ = 17α(H)21β(H), βα = 17β(H)21α(H), S and R indicates stereochemistry at the C-22 position.

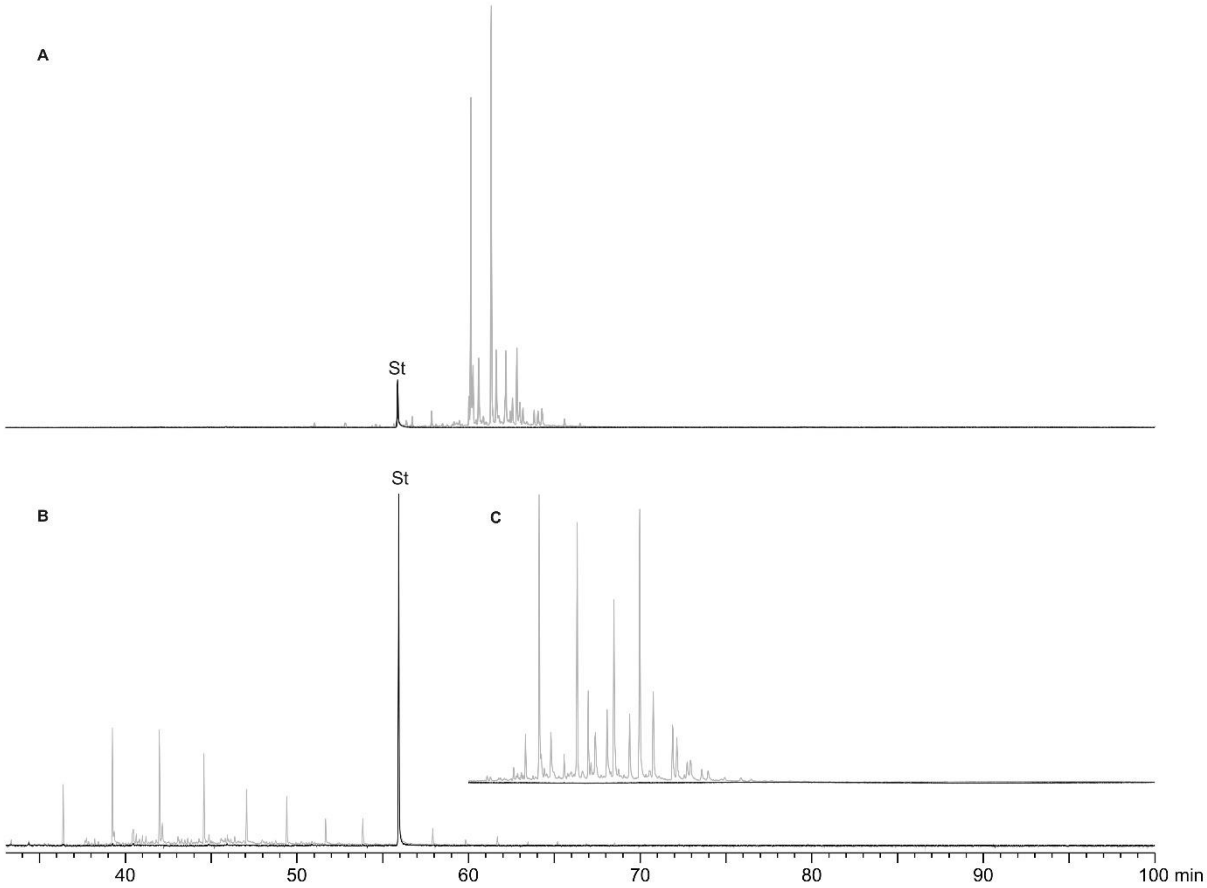

Fig. S6 | GC-MS chromatograms of a comprehensive accumulatory laboratory system blank (black line) in comparison to a *Dickinsonia*-2 extract (grey line). (A) Selected Ion Recording (SIR) chromatogram showing monoaromatic steroids on mass trace *m/z* 253 ; (B) full-scan chromatogram *m/z* 85 trace (*n*-alkanes and methylalkanes); (C) Metastable Reaction Monitoring (MRM) chromatogram: Σ($C_{27–35}$ hopanes, $C_{27–29}$ steranes); St: 18-MEAME internal standard.

	MAS_y/H[1]			5β/5α[2]		
	C_{27}	C_{28}	C_{29}	C_{27}	C_{28}	C_{29}
***Dickinsonia*-1**	0.155 *(0.004)*[5]	0.022 *(0.001)*	0.147 *(0.003)*	3.606 *(0.207)*	0.519 *(0.324)*	0.600 *(0.057)*
***Dickinsonia*-2**	0.254 *(0.005)*	0.028 *(0.001)*	0.145 *(0.004)*	5.490 *(0.28)*	0.652 *(0.231)*	0.754 *(0.056)*
***Dickinsonia*-3**	0.156 *(0.004)*	0.025 *(0.001)*	0.148 *(0.004)*	4.079 *(0.29)*	0.749 *(0.265)*	0.628 *(0.043)*
***Dickinsonia*-4**	-	-	-	2.656 *(0.408)*	0.972 *(0.344)*	0.635 *(0.08)*
***Dickinsonia*-5**	-	-	-	2.329 *(0.102)*	0.814 *(0.288)*	0.447 *(0.019)*
***Dickinsonia*-6**	-	-	-	1.337 *(0.071)*	0.788 *(0.279)*	0.500 *(0.022)*
Variance (*10^{-4})[3]	32.617 *(0.007)*	0.076 *(0.001)*	0.024 *(0.005)*	21379 *(4.4)*	235 *(5.038)*	117 *(0.878)*
***Dickinsonia* sterol composition**[4]	99.70% *(0.33)*	0.23% *(0.04)*	0.07% *(0.16)*	98.38% *(0.04)*	1.08% *(0.02)*	0.54% *(0.004)*

Table S1. Estimation of the sterol composition of *Dickinsonia.*

[1] Concentration of C_{27}, C_{28} and C_{29} monoaromatic steroid (MAS) homologs (sum of isomers I and V) relative to total hopanes (sum of C_{27}–C_{35} hopanes) in *Dickinsonia* extracts; 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); y indicates carbon number of monoaromatic steroids; [2]5β/5α = (βααα20R+αααα20S)/αααα20R; [3]Variance = $\sum(x-\bar{x})^2/(n-1)$, where x is C_y/H value, $\bar{x}$ is a mean C_y/H value between the samples and n is the number of samples (n = 3); [4]C_y% = 100%*(C_y variance)/(sum of variances for each of three steroids), y indicates carbon number of monoaromatic steroids; [5]numbers in parentheses are standard deviation values.

	C_{27}	C_{28}	C_{29}
***Andiva*-1 5β/5α[1]**	1.072 *(0.036)*[4]	0.758 *(0.268)*	0.685 *(0.017)*
***Andiva*-2 5β/5α[1]**	1.315 *(0.150)*	0.811 *(0.287)*	0.517 *(0.042)*
***Andiva*-Mat 5β/5α[1]**	0.805 *(0.068)*	0.761 *(0.269)*	0.65 *(0.041)*
Variance ($*10^{-4}$)[2]	651.363 *(1.192)*	8.938 *(3.369)*	78.694 *(0.432)*
***Andiva* sterol composition[3]**	88.14%[5] *(0.46)*	1.21% *(0.46)*	10.65% *(0.08)*

Table S2. Estimation of sterol composition of *Andiva*.

[1]5β/5α = (βαα20R+ααα20S)/ααα20R; [2]Variance = $\sum(x-\bar{x})^2/(n-1)$, where x is C_y/H value, $\bar{x}$ is a mean 5β/5α value for each sterane between the samples and n is the number of samples (n = 3); [3]C_y% = 100%*(C_y variance)/(sum of variances for each of three steroids); [4]numbers in parentheses are standard deviation values; [5]conservative minimum estimate of relative cholesteroid content (C_{27}%) of *Andiva* (see Supplementary Text).

Sample	Ts/(Ts+Tm) C_{27} hopanes	βα/(βα + αβ) C_{30} hopanes	22S/22(S+R) C_{31} hopanes	C_{27-30}/C_{31} hopanes[1]	2-MHI (%)[2]	3-MHI (%)[3]	S/H[4]
***Dickinsonia*-1**	0.09 *(0.01)*	0.24 *(0.01)*	0.57 *(0.01)*	0.41 *(0.01)*	5.22 *(0.03)*	0.82 *(0.08)*	1.92 *(0.16)*
***Dickinsonia*-2**	0.08 *(0.00)*	0.27 *(0.01)*	0.55 *(0.01)*	0.59 *(0.01)*	6.44 *(0.03)*	0.91 *(0.09)*	1.39 *(0.13)*
***Dickinsonia*-3**	0.09 *(0.01)*	0.24 *(0.01)*	0.58 *(0.01)*	0.38 *(0.01)*	5.57 *(0.03)*	0.96 *(0.11)*	2.57 *(0.24)*
***Dickinsonia*-4**	0.09 *(0.01)*	0.27 *(0.03)*	0.56 *(0.03)*	0.26 *(0.01)*	6.32 *(0.06)*	1.00 *(0.22)*	1.91 *(0.34)*
***Dickinsonia*-5**	0.11 *(0.01)*	0.27 *(0.02)*	0.55 *(0.02)*	1.41 *(0.04)*	6.15 *(0.06)*	-	-
***Dickinsonia*-6**	0.09 *(0.01)*	0.27 *(0.02)*	0.55 *(0.02)*	0.89 *(0.02)*	6.22 *(0.05)*	-	-
***Dickinsonia*-7**	0.08 *(0.02)*	0.32 *(0.06)*	0.53 *(0.06)*	0.73 *(0.05)*	5.93 *(0.12)*	-	-
***Dickinsonia*-8**	-	-	-	-	-	-	-
***Dickinsonia*-Sandstone**	0.10 *(0.00)*	0.25 *(0.01)*	0.53 *(0.01)*	0.44 *(0.01)*	4.48 *(0.02)*	1.13 *(0.07)*	2.88 *(0.15)*
***Dickinsonia*-Clay**	0.07 *(0.00)*	0.31 *(0.01)*	0.55 *(0.02)*	0.27 *(0.00)*	3.85 *(0.87)*	1.47 *(0.07)*	2.89 *(0.13)*
***Andiva*-1**	0.03 *(0.00)*	0.31 *(0.01)*	0.38 *(0.01)*	0.17 *(0.00)*	3.82 *(0.02)*	0.95 *(0.08)*	1.34 *(0.12)*
***Andiva*-2**	0.10 *(0.01)*	0.30 *(0.03)*	0.40 *(0.03)*	0.17 *(0.01)*	3.93 *(0.05)*	1.76 *(0.27)*	1.06 *(0.21)*
***Andiva* Mat**	0.07 *(0.01)*	0.33 *(0.03)*	0.37 *(0.03)*	0.19 *(0.01)*	3.82 *(0.05)*	1.11 *(0.21)*	1.29 *(0.26)*
***Andiva*-Sandstone**	0.04 *(0.00)*	0.37 *(0.01)*	0.39 *(0.02)*	0.24 *(0.00)*	5.63 *(0.02)*	0.80 *(0.06)*	0.93 *(0.08)*
***Andiva*-Clay**	0.05 *(0.00)*	0.33 *(0.01)*	0.38 *(0.01)*	0.22 *(0.00)*	3.03 *(0.02)*	0.96 *(0.05)*	0.68 *(0.04)*

Table S3. Hopane and methylhopane biomarker parameters in extracts of dickinsoniids and surrounding deposits.

[1]C_{27} = Σ(Ts, Tm, β), C_{29} = Σ(αβ, Ts, βα), C_{30} hopane = Σ(αβ, βα), C_{31} = Σ(αβ-22(S+R), βα); [2]2-methylhopane index, 2-MHI (%) = 100*(Σ C_{31} 2α+2β-methylhopane)/ (Σ C_{31} 2α+2β-methylhopane + Σ(C_{30} αβ-22(S+R), βα hopane); [3]3-methylhopane index, 3-MHI (%) = 100*(Σ C_{31} 3β-methylhopane)/ (Σ C_{31} 3β-methylhopane + Σ(αβ-22(S+R), βα C_{30} hopane); [4]S/H = Σ(C_{27-29} steranes)/Σ(C_{27-35} hopanes), steranes: C_{27} = Σ(βα-20(S+R)-diacholestane, ααα- and βαα-20(S+R)-cholestane), C_{28} = Σ(βα-20(S+R)-diaergostane, ααα- and βαα-20(S+R)-ergostane), C_{29} = Σ(βα-20(S+R)-diastigmastane, ααα- and βαα-20(S+R)-stigmastane), ααα = 5α(H),14α(H),17α(H), βαα = 5β(H),14α(H),17α(H); 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); [5]numbers in parentheses are standard deviation values.

Species	Type	Sample Origin	C_{27} to C_{29} Sterols		
			C_{27}%	C_{28}%	C_{29}%
Collodaria SpB	Polycystinea (Radiolaria)	Mediterranean (Villefranche sur Mer)	75.38	16.10	8.52
Collodaria SpA	Polycystinea (Radiolaria)	Mediterranean (Villefranche sur Mer)	73.82	18.77	7.41
Collodaria SpC	Polycystinea (Radiolaria)	Mediterranean (Villefranche sur Mer)	75.70	15.17	9.13
Collodaria SC2	Polycystinea (Radiolaria)	Mediterranean (Villefranche sur Mer)	60.54	23.30	16.16
Collodaria SC1	Polycystinea (Radiolaria)	Mediterranean (Villefranche sur Mer)	61.86	14.50	23.64
A. lobifera	Foraminifera	Red Sea (Eilat)	13.58	43.16	43.26
Sorites sp.	Foraminifera	Red Sea (Eilat)	30.67	20.06	49.27
Shepheardella sp.	Foraminifera	Red Sea (Eilat)	45.72	16.16	38.12
Quinqueloculina sp.	Foraminifera	Mediterranean (Tel Aviv)	78.15	14.66	7.19
Acantharea sp.	Acantharea	Indian Ocean near Eden	51.98	39.35	8.67
Gromia sp.	Gromiidae	Southern Ocean (McMurdo Sound)	11.77	27.88	60.35
A. laticollaris[1]	Foraminifera	Literature (*31*)	10.31	26.93	62.76

Table S4. Distribution of C_{27}-C_{29} sterols in extant Rhizaria (Retaria and Gromiidae)

[1]Values for *A. laticollaris* were determined by integrating peak areas of the functionalized sterols in Fig.1a of Grabenstatter et al. (*31*).