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Supplementary Materials for

Cryogenian evolution of stigmasteroid biosynthesis

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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- table S1 (.pdf format). Steranes in Neoproterozoic rocks.

Supplementary Text

1. Contamination control and steroidal signature verification

In recent years it has become evident that hydrocarbon contamination from ancient, modern and/or anthropogenic sources can pose a significant problem when analyzing samples of ancient rocks *(12–14, 32, 34, 35, 36)*. To overcome ambiguities and establish whether detected hydrocarbons are syngenetic and indigenous, solely indigenous (but not syngenetic) or derive from contamination introduced during residence, sampling or during the laboratory workup process, we separated all samples into interiors and exteriors, and analyzed these in parallel with procedural blanks. Sample/procedural blank ratios were calculated for relevant analytes and where lean samples were deemed to have been contaminated in the laboratory, these were excluded form consideration. In practice however, the more discriminating approach involved comparing sample interiors and exteriors. Where samples were deemed contaminated, we commonly also observed that 'lean' or empty sample interiors fell into the exclusion window in comparison to procedural blanks, whereas this was rarely the case for sample exteriors. As previously detailed by Brocks and colleagues (*12, 37*) the distribution of hydrocarbons and (if present) plastic-derived contaminants can frequently reveal if samples are partially (i.e. externally) or pervasively contaminated. Such comparisons showed no significant differences for most samples that were used in this study. However, where plastic-derived contaminants had pervaded into sample interiors, or where exterior and interior hydrocarbon signatures were identical but significantly different in concentration, we decided to exclude these samples from further consideration. Additional care was taken when interpreting interior hydrocarbon signatures. Some samples contained typical plant-derived biomarkers (e.g. retene), even though said samples did not display apparent contamination as described above, and would have fitted the general steroidal patterns. Nevertheless these samples (in this specific case, those from the upper Ediacaran Lublin Fm, Poland: table S2) were also excluded from consideration. Likewise, the steroidal signature was scrutinized in both the saturated hydrocarbon fraction (i.e. saturated steranes) and the aromatic hydrocarbon fraction (aromatic steroids). Where saturated and aromatic steroids harbored different compositions, aromatic steroids were given

preference when considering syngeneity, since they were likely produced through maturation during burial and are slightly less likely to be affected by external migration. In some samples the saturated steroids were a mixture of both syngenetic and allochthonous (i.e. migrated) molecules, and these samples were hence also excluded.

In Section 4 of these Supplementary Materials we document the total number of samples used from every setting, whereas table S1 only lists those samples, in which the hydrocarbon signature can be confidently assigned as certainly indigenous. Some of the settings, listed in Section 5, yielded not a single sample with a reliably indigenous hydrocarbon signature. These samples were either insufficiently organic-rich, too thermally mature (i.e. indigenous hydrocarbons present, but no surviving steranes), or contaminated and hence have not been considered in arriving at the conclusions of this study.

2. No diagenetic, maturity or generation control on relative sterane abundances

The relative proportion of C_{27}:C_{28}:C_{29} steroids in primary producing biomass is directly related to that found in ancient sediments and is not skewed by any secondary processes. The conversion of sterols (i.e. stenols) to steranes, via stanol and sterene intermediates, has received much scientific attention and is by now relatively well understood (e.g. *38, 39*). This conversion essentially involves defunctionalization and reduction, and does affect all sterols in a similar manner: none of the processes modify the side-chain at position C-24 (*38, 39*). The generation of bitumen or petroleum from a kerogen phase also does not, or only marginally, affect sterane distributions. We subjected two fully-extracted samples of the Masirah Bay Fm to catalytic hydropyrolysis, thereby releasing steranes *de-novo* from the kerogen. Here, the largest observed offset involved C_{27}:C_{28}:C_{29} values of 21 : 22 : 57 in bitumen and 23 : 34 : 43 in kerogen. Similarly, the relative abundance of these three major steranes does not (*40, 41*)—or only negligibly (e.g. *42, 43*)—change with increasing thermal maturity. Furthermore, it was recently shown in an

artificial maturation study that the overall relative distribution of C_{27}:C_{28}:C_{29} steranes remains more or less constant throughout all maturity stages and up to the thermal destruction of steranes (*44*), which was achieved by heating for 5 hours at 400ºC (but notably not 2400 hours at 300ºC). This provides solid support for the originality of the steroid distributions observed in this study.

While biodegradation may mildly alter the C_{27}:C_{28}:C_{29} sterane ratio, we did not detect any evidence of such alteration in any of the studied samples. Biodegradation starts in the lower-molecular-weight range targetting readily degradable straight-chain hydrocarbons (*viz. n*-alkanes). Only at advanced stages of biodegradation are polycyclic terpenoids such as hopanoids and steroids attacked (*41*). By then all *n*-alkanes will have been removed, resulting in a concomitantly larger abundance of branched and cyclic alkanes, including an enhanced presence of biodegradation products such as 25-norhopanes. Yet none of our samples displayed such molecular signatures. Also, no significant chromatographic humps of UCM (unresolved complex mixture), typically indicative for anhanced biodegradation, were encountered in samples that contributed to the conclusions in this study. Therefore, the detected sterane proportions were concluded to retain the unaltered composition of their precursor biomass. No other secondary process can modify the C_{27}:C_{28}:C_{29} sterane distribution in a way that would affect our conclusions.

3. Cryogenian divergence of C_{29} sterol methylation genes in green algae

As steroid biosynthesis is increasingly better understood (e.g. *4*) new avenues are opened that allow the use of comparative genetics in order to generate time-calibrated phylogenetic trees depicting the origin of certain biochemical traits. Gold and colleagues (*30*) have created such a tree in view of shedding more light on the rise of 24-isopropylcholesterol biosynthesis. This tree was based on genes coding for sterol methyltransferase enzymes (*carbon-24/28-sterol methyltransferase*) thought responsible

for methylation at C-24. Their dataset includes the prasinophyte green alga, *Ostreococcus*, which contains two sterol methyltransferases (SMT) considered responsible for alkylation at position C-24. We confirm the existence of exactly two SMT genes also in the prasinophyte green algae *Chlamydomonas reinhardtii*, *Bathycoccus prasinos* and *Micromonas pusilla CCMP1545*, whose genomes are available in public databases as of May 2017. In the phylogenetic tree constructed on the basis of SMTs (Fig. 3 in (*30*)) the two green algal SMTs (Ostr_ABO05008 and Ostr_ABO97661) diverge from their common ancestors *exactly* at the end of the Cryogenian Period (fig. S1). This aspect of SMT evolution was not discussed by Gold and colleagues (*30*) but provides independent evidence for a Cryogenian split in green algal steroid methylation. This is consistent with our observations on the appearance of C_{29} steranes in the rock record and provides independent support for the late Cryogenian evolution of stigmasteroid biosynthesis in an ancestral green alga.

4. Samples with uncontaminated interior biomarker signatures

In this section we list those stratigraphic units, of which at least one sample was deemed to contain indigenous and uncontaminated steranes in its interior portion. The total number of analyzed samples is listed with a brief review and explanation of its geological setting and age bracket. The modern day localities of samples are shown in fig. S2, whereas fig. S3 shows an annotated version of Figure 1. A ✖ in the title implies that these samples were worked up and analyzed *de novo* in our laboratory, whereas a ⭘ indicates formations with literature derived data.

1. Liulaobei Fm (3 samples): 900–800 Ma ✖

Samples from the Liulaobei Fm were provided by Qing Tang and Shuhai Xiao. They were collected in the field (Diangeda-Baieshan section: 32°37′51.52″N, 116°46′0.19″E) in 2005 and 2012. The Huainan Group (containing the Bagongshang and Liulaobei Fms)

unconformably overlies Mesoproterozoic crystalline basement, and is further constrained by two K-Ar dates of 738.6 and 749.8 Ma in the conformably overlying Shouxiang Fm (*45*, *46* and references therein). An 840 Ma intra-Liulaobei Rb/Sr date is corroborated by the occurrence of the acritarch *Trachyhystrichosphaera aimika* (*47*), which is inconsistent with a Cryogenian or younger age (*48*) and definitely places the Liulaobei Fm into the ~900–750 Ma age bracket, given the stratigraphy (*46*) more likely in the range 900–800 Ma.

2. Kanpa Formation (8 samples): 820–720 Ma ✖

Core samples of the Kanpa Fm, Officer Basin, recovered from drill hole Empress-1 located in the western Officer Basin (27º03’13”S, 125º09’24”E), were collected at the Geological Survey of Western Australia, Perth. Of eight studied samples, only three contained uncontaminated indigenous biomarker hydrocarbons in their interior portion. The sedimentary supersequence containing the Kanpa Fm (Browne, Hussar and Kanpa Fms) is unconformably underlain by crystalline basement with a K-Ar date of 1058 ± 13 Ma, and unconformably overlain by coarse clastics of the Lupton Fm, which has been dated to 791 ± 18 Ma (Pb/Pb) and 788 ± 26 Ma (Pb/U) using detrital zircons (*49*). The Kanpa Fm is constrained to a late Tonian age (ca. 820–720 Ma: *50*, *51*) by the first occurrence of the acritarch *Cerebrosphaera buickii* in the conformably underlying Hussar Fm, as well as by the presence of *Chuaria circularis* within its strata (*49*).

3. Bitter Springs Fm: 780–770 Ma ⭕

An indigenous C_{27}-only sterane signature was identified in the ~770 Ma (*52*) Johnny’s Creek Member of the Bitter Springs Formation, Amadeus Basin, central Australia by Jarrett (*53*).

4. Kwagunt Fm (57 samples): 755–740 Ma ✖

Samples from the Kwagunt and Galeros Fms of the Chuar Group were collected on the north flank of Nankoweap Butte (36º16’22”N, 111º53’29”W), Grand Canyon, Arizona, USA in 2014. Only the Kwagunt Fm (28 samples) has yielded indigenous steroids. The principal age control comes from a tuff bed overlying the Kwagunt Fm, which has yielded a zircon age of 742 ± 6 Ma (*54*) and the occurrence of *Chuaria circularis* within its strata—confirming the Late Tonian age. Given the occurrence of steranes in ca. 300 m of shale that accumulated prior to the deposition of the tuff, we can assign an approximate age bracket of 755–740 Ma by assuming an exceptionally conservative low sediment accumulation rate of 20 m / Myr (*55*).

5. Visingsö Group (11 samples): 788–740 Ma ✖

Samples from the Visingsö Group were collected on Visingsö Island (Ed and Näs localities), as well as on the eastern shore of Late Vättern (Boeryd, Omberg, Girabäcken localities) in June 2016. The maximum age of the Visingö Group was constrained to 886 ± 9 Ma by detrital zircon analyses (*56*), while microfossils and acritarchs, including amongst others *Chuaria circularis*, *Cerebrosphaera globosa* and notably vase-shaped microfossils are consistent with a Tonian age (*57–59*). On the basis of the occurrence of mineralized scale microfossils, the age range of the Middle and Upper Visingsö Group, where our sample derive from, is most likely constrained to the period 788–740 Ma (*56*).

6. Mirassol d’Oeste Fm (23 samples): 635 Ma ✖

The Mirassol d’Oeste Fm was sampled by Pierre Sansjofre in 2009. Multiple closely spaced quarries were targeted, but only seven samples from the Terconi quarry (15º40’42”S, 58º04’32”W) and one sample from the Tangara quarry (14º39’25”S, 57º31’22”W) contained indigenous uncontaminated steranes. This cap dolostone was constrained to belong to the Marinoan deglaciation (~635 Ma) by its sedimentological features (*60*), $^{87}Sr/^{86}Sr$ values between 0.7074 and 0.7085 (*61*), as well as by Pb/Pb dates

of 627 ± 32 Ma and 622 ± 33 Ma (*62*) that were obtained on samples of the lowest Guia Fm, which directly and comformably overlies the Mirassol d'Oeste Fm.

7. Koniakari Group (4 samples): 635 Ma ✖

Samples from the cap carbonate of the Koniakari Group (K1/28: 14º35'37.39''N, 10º52'29.24''W) and the overlying black cherts of the Nioro Group (K1/31: 14º36'44.76''N, 10º50'46.85''W) were collected from exposures of the Kaarta Mountains, near Kayes, Mali. The Neoproterozoic glaciogenic 'triad' (tillite-cap carbonate-silexite) cropping out in the Hohd sub-basin of the Taoudéni Basin (*63*) was deposited between the Bassaride (665–655 Ma) and Dahomeyide (610–580 Ma) orogens (*64*). This bracket allows assignation of the Koniakari cap carbonate to the end of the late Cryogenian (Marinoan) glaciation, which fits with two confident U-Pb zircon and SHRIMP ages obtained by the analysis of tuffites that overlie the cap carbonate in the Adrar of Mauritania and the Mauritanide Belt (*65–69*).

8. Chistyakovskaya and 10. Oskobinskaya Fm (5 samples): 620–600 and 600–560 Ma ✖

Shales and carbonates of the Chistyakovskaya Fm, which comprise the second lowest stratigraphic unit of the Ediacaran Taseeva Group (*70*), conformably overlie coarser siliciclastics of the Alyoshinskaya Fm, which in turn discordantly overlies Lower Neoproterozoic metamorphic sediments of the Tungusik Group (*71*). Recently a diamictite (Shishina Member) was discovered in some localities at the base of the Taseeva Group (*71*), suggesting an earliest Ediacaran age for the Alyoshinskaya Fm. Drill hole Solzavodskaya-1 is located in the southern Yenisey Ridge area and recovered the Taseeva Group and overlying sediments from the Irkineevo-Chadobetsky basin. Around 150 km north of this locality, a correlative siliciclastic and carbonate succession (Chapa river section) was studied by Pokrovsky and colleagues (*72*). Here, diamictites of the Chivida Fm can likely be correlated with those at the base of the Taseeva Group. Furthermore, a minimum $^{87}Sr/^{86}Sr$ value of 0.7076 in carbonates of the Podyom Fm, ca. 90–120 m above the diamictite, point to an age close to that of the Marinoan deglaciation

(*73*). A spike in negative stable carbon isotope values around –8 permil between the Podyom Fm and another $^{87}Sr/^{86}Sr$ value of 0.7084 suggest that this might be a correlative of the Shuram isotope anomaly. What this means is that by lithostratigraphic correlations between the Chapa river section and the sequence recovered by drill hole Solzavodskaya-1, the Chistyakovskaya Fm is most probably of very early Ediacaran age and is here assigned a tentative age bracket of 620–600 Ma. While thermally mature and relatively organic lean, one of the studied core samples yielded indigenous steranes with a C_{27} predominance.

The Oskobinskaya Fm was studied in core samples from drill hole Kamovskaya-1, located in the same area but towards the northern edge of the Irkineevo-Chadobetsky basin, therefore recording a much more condensed section. In Kamovskaya-1, the Oskobinskaya Fm is situated about 200 m below evaporites that can be correlated to the early Cambrian Terreneuvian series (Tommotian: 534–530 Ma) Usolye Fm (*74*). It should be noted that along the basin edge, early Ediacaran erosional processes were active, through which the lower part of the Taseeva Group (i.e. Chistyakovskaya and Alyoshinskaya Fms) was removed. Hence a stratigraphic distance to the base of the Ediacaran cannot be measured. However, this can be done in the correlative drill hole Beremichenskaya-1, where the stratigraphic distance of concordant strata between the base of the Oskobinskaya Fm and the base of the Taseeva Group amounts to ca. 550 m, while the stratigraphic distance between the top of the Oskobinskaya Fm and the bottom of the Usolye-correlative evaporites is ca. 1000 m. This suggests that in the absence of any better age constraints, we can place the Oskobinskaya Group within the middle Ediacaran and hence we again assign to it a wide age bracket of 600–560 Ma, which must undoubdedly capture the short interval of Oskobinskaya deposition. We would like to point out that for the purpose of this study, where steroidal abundances are discussed in the framework of four rather broad time windows (Fig. 1), this imprecise age date is not as crucial as the molecular data. The latter are highly interesting, since in contrast to the slightly older Chistyakovskaya Fm, the Oskobinskaya Fm carries a C_{29} steroidal dominance.

9. Masirah Bay and Khufai Fms (5 and 1 samples): 635–613 Ma and 613–580 Ma

Rocks from the SOSB, Oman, are exceptionally bitumen-rich and, unusually for their age, thermally immature. The data used in this study comes from a comprehensive petroleum geochemical investigation of the SOSB (*75*) and were verified by repeat analysis of two of the original samples. These yielded C_{27}/C_{29} sterane values of 0.38 and 0.12 in our laboratory, as compared to the published values of 0.30 and 0.10 (*75*). Given this congruence, we had no reservation in using the published data. Calcareous shales of the Masirah Bay Formation conformably overlie the Marinoan-age Hadash cap carbonate (which was rapidly deposited (*76*) and in turn overlies the Fiq diamictite) and hence must be up to 635 Ma in age (*55*). The Masirah Bay Fm is conformably overlain by the Khufai Fm, which in turn is separated by an unconformity from the Shuram Fm. Although it is not known what amount of time is missing from the sedimentary record due to a paucity of absolute ages, an age bracket can be inferred by interpolation. The Masirah Bay and Khufai carbonate carbon stable isotopic record lies stable between 0 and 4 ‰ VPDB (*77*) (compare to the calibrated $\delta^{13}C$ record presented by (*78*)), implying that the negative isotopic signature that is associated with the regional Gaskiers glaciation (*79*) is not recorded in the SOSB. The deposition on the Avalon Peninsula of low-latitude diamictites (*80*) associated with this glaciation, during which the Earth likely narrowly escaped a third Snowball event (*81*), were recently constrained to a short duration between 580.9 Ma and 579.9 Ma. Hence the Masirah Bay–Khufai couplet should occupy a 635–580 Ma age bracket. However, given continuous sediment deposition and the stratigraphic thicknesses of both formations (visually estimated to be ca. 180 m of Masirah Bay *vs*. ca. 270 m Khufai in drill hole Miqrat-1: (*55*)), the Masirah Bay Fm is more likely to be constrained to 635–613 Ma and the Khufai Fm to 613–580 Ma — but only if the top of the Khufai corresponds to an age of 580 Ma, which is unlikely. Nevertheless, we decided to use these age brackets given that the Masirah Bay Fm clearly largely falls within the early Ediacaran age bracket, whereas a large portion of the Khufai Fm probably corresponds to the early part of the Middle Ediacaran bracket.

A dominance of C_{29} over C_{27} steranes was found in deep-water carbonates of the Khufai Fm, in which the organic matter was deemed to be marginally mature on the basis of its biomarker stereochemistry and Rock Eval T_{MAX} values <440ºC (*82*). Given the generally good preservation and high abundances of immature bitumen in the SOSB (*83*), we have no doubt about the indigeneity of this signature.

10. Oskobinskaya Fm ✖

See: 8. Chistyakovskaya Fm.

11. Krol A Formation (1 of 16 samples): 590–562 Ma ✖

A suite of samples from the Krol Group was collected during 2013 in the Mussoorie syncline (lesser Himalaya, Uttarakhand, India) along Chamba Road leading from Mussoorie to Dhanolty. Most samples were strongly weathered and thereby contaminated. One fresh sample (M4, collected at 30º26'09.4"N, E78º13'28.5"E ± 5m at 2338 m altitude), a pyritic black shale from the Krol A stratigraphic level (*84*) was obtained from a recent cut in the roadside and yielded indigenous terpenoids. While no radiometric ages have been determined, the Kroll Group is bracketed by the Cryogenian Blaini diamictite and the overlying Cambrian Tal Group, hence constraining it to the Ediacaran (*84*). Despite enhanced scatter, most likely due to a slight diagenetic overprint, a detailed carbon isotope stratigraphic record has been obtained from the Infra-Krol Fm and Krol Group (*85*). A composite record after selection for least altered samples still reveals one major negative carbon isotope excursion (ca. from +4 to -10 ‰ over ca. 150 m) in the Krol B unit that likely reflects a condensed equivalent of the global Shuram isotopic anomaly, which has been dated at ca. 562–552 Ma in the South Oman Salt Basin (*77*). The Krol A unit (represented by sample M4) directly underlies this isotopic anomaly in the Mussoorie syncline and hence should be 'slightly older' than 562 Ma, yet significantly younger than the Marinoan cap carbonate (~635 Ma). We therefore assign sample Mussoorie M4 a broad age bracket with an estimated range of 590–562 Ma.

12. Dey Dey Mudstone (7 samples): ca. 580–560 Ma

While no absolute age dates exist for this formation in the eastern Officer Basin, it records the Acraman impact event (*86*), the characteristic ejecta of which can be used as a chronostratigraphic marker to correlate this stratigraphic unit to the Bunyeroo and Yarloo Formations in the adjacent Adelaide Fold Belt, where the latter unit has a Rb/Sr whole-rock age of 588 ± 35 Ma (*87*). The Bunyeroo Fm conformably underlies the Wonoka Fm, which records a Shuram-equivalent isotope anomaly (*88*), thus making the Bunyeroo Fm and Dey Dey Mudstone older than 554–562 Ma (see section on the Shuram Fm), yet younger than ~580 Ma. This is in agreement with a $^{87}Sr/^{86}Sr$ value of 0.7079 in the Karlaya Limestone (conformably overlying the Dey Dey mudstone) (*89*), which corresponds to a plateau in the global Neoproterozoic $^{87}Sr/^{86}Sr$-curve that corresponds to the ca. 600–580 Ma time interval (*73*). Given that all the Dey Dey values used in this study (see below for rationale of choice) come from stratigraphic levels above and closeby the Acraman ejecta layer, we here assign them an age bracket of 580–560 Ma.

The hydrocarbon inventory of the Dey Dey Mudstone and overlying Karlaya Fm has been documented in the context of the Acraman impact event (*90*). Many of the studied samples are rather organically lean. Accordingly, we have re-analyzed their data and selected only the richest samples for use in this study. The total organic carbon (TOC) content and abundance of extractable organic matter (EOM) correlate linearly with an R_2 value of 0.61, albeit with quite a bit of scatter. Experience has shown that organically leaner samples are more susceptible to receiving a contaminant overprint. The key contamination sources for drill cores are the fluids employed during drilling and plastics during storage; and their signatures commonly affect cores from the same drill hole in a similar fashion, but to an extent that depends on the concentration of indigenous organic matter. Hence we have here decided to only use samples that fulfil two criteria: TOC > 0.1 % *and* EOM > 100 ppm. As a result, only seven of the 58 samples examined by McKirdy and colleagues (*90*) from drill holes Munta-1 and Observatory Hill-1 are used in the current compilation.

13. Shuram and 14. Buah Fms (9 samples): 562–552 Ma and 552–547 Ma

Age constraints on this upper part of the Nafun Group remain imprecise due to a lack of direct radiometric dates. While the contact between the Shuram Fm and the overlying Buah Fm is conformable, this package is separated by hiati from the underlying Khufai Fm and the overlying Ara Group. In the latter, the middle of the A0 unit (basal Ara Group) has been dated at 546.7 Ma, thereby providing an absolute minimum age (*55*), whereas a maximum age of ~580 Ma is constrained by the Khufai-Shuram hiatus and the absence of a characteristic Gaskiers carbon isotope signature (see age discussion for the Masirah Bay Fm). This notion is supported by a detrital zircon age from a shale level, ~5 m above the base of the Shuram Fm, which yielded a youngest maximum age of 620 Ma (*55*). A mid-Khufai age of 550.5 Ma was inferred on the basis of correlating the Shuram isotopic recovery in the SOSB to that in China, with the underlying assumption that these anomalies not only share the same mechanistic driver but are also synchronous in both onset and recovery. The stratigraphic level where the Shuram anomaly first crosses the -3 ‰ VPDB post-nadir mark was correlated to the same level in the Doushantuo Fm, which carries a U-Pb date of 550.5 ± 0.8 Ma at this stratigraphic position (*91*). Furthermore, a linear interpolation of sediment thickness between dated horizons (both directly dated and reconstructed by stratigraphic inference) that assumes depositional continuity, allowed Bowring and colleagues (*55*) to assign an age of 554–562 Ma to the basal strata of the Shuram Formation. We here use the same approach and data (40–90 m/Myr deposition, 68.7 m sediment thickness between the Shuram-Buah boundary and the stratigraphic level of the inferred 550.5 Ma date in well Miqrat-1; reconstructed from (*55*): Figure 10) and calculate an age between 551.26 and 552.22 Ma for the Shuram-Buah boundary. Hence we use the more generous age bracket of 562–552 Ma for the Shuram Fm and a bracket of 552–547 Ma for the Buah Fm.

14. Buah Fm

See: 13. Shuram Fm.

15. Salt Range Formation (8 samples): ca. 550–540 Ma

During the late Neoproterozoic and early Cambrian (Terreneuvian series), a wide continental shelf extended all along the Arabian-African margin, containing a series of correlative sedimentary depocenters including the Hormuz (Iran), South Oman, South Punjab/Nagaur-Ganganagar Basins that form a distinct elongate ‘salt basin domain’ extending across present day Oman, Saudi Arabia, Iran, Pakistan and India (*92*). This implies that the Salt Range Formation of Pakistan is most likely age equivalent to the Hansean (evaporite suite) Group in India and the Ara Group in Oman (*92*). Given the exceptionally immature and bitumen-rich nature of these samples we have no doubt about the syngenicity of the reported steroidal distributions (*93*).

16. Baghewala oil (10 samples): ca. 550–540 Ma

Oil shows and oil-stained sandstone units in the Baghewala-1 well, Bikaner-Nagaur Basin, India, were generated by source rocks in the Marwar Supergroup, which spans a latest Neoproterozoic to Terreneuvian age (*94*). In this area, a crystalline basement (Malani Rhyolite, ca. 740 Ma: (*95*)) is uncomformably overlain by sandstones and coarse siliciclastics of the Jodhpur Group, Bilara Group carbonates, Hanseran evaporites and the Nagaur Group. Geochemical studies have correlated the oils to a marginally mature source rock in the Bilara Group (*96*) where oil-prone source intervals with elevated TOC and high hydrogen indices (HI; up to 400 mg HC/g TOC) are present at 1068–1120 m depth in drill hole Baghewala-1 (*95*). The sterane data employed in this study were obtained by Dutta and colleagues (*97*). The Bilara Group and overlying Hanseran evaporite sequence are considered the stratigraphic equivalent of the Pakistani Salt Range and the Omani Ara Group (*98*), which were deposited in a series of correlative depocenters on a wide continental shelf extending all along the Arabian-African margin (*92*). Hence we assign the Baghewala oils to the 550–540 Ma age bracket.

17. Gavrilov Jam well (2 samples): ca. 550–540 Ma

A C_{29}-sterane dominance was reported from bitumens extracted from Ediacaran rocks in the Gavrilov Jam drill hole, located in the Moscow Syncline (*99*). Given the low thermal maturity (T_{MAX} <435ºC), oil-prone (up to 260 mg HC/g TOC) character and high EOM yields of these rocks, we have no doubts about the syngeneity of their reported hydrocarbons. A Vendian age constraint (Noginskaja, Redkinskaja and Povarovskaja Fms) is supported by the geological succession and by biostratigraphic correlation of these units to the White Sea strata. We (i.e. Małgorzata Moczydłowska) have analyzed the microfossil content of these Gavroliv Jam samples and found *vendotaenids*, *Leiosphaeridia*-type organic-walled microfossils, as well as *Sabellidites*. In particular the latter can be correlated to the terminal Ediacaran microfossil succession of the White Sea and other areas on the East European Platform, thereby constraining the age to be upper Ediacaran to lowermost Cambrian (*100, 101*). We hence confidently assign an age bracket of 550–540 Ma.

18. Khatyspyt Formation (1 sample): ca. 551–544 Ma

Ediacara-type macrofossils in the Khatyspyt Fm place it in the latest Ediacaran. The unit is comformably overlain by the Turkut Fm, where a U-Pb date of 543.9 ± 0.3 (*102*) has been obtained from a volcaniclastic breccia. The upper part of the Turkut Fm contains the first occurrence of the shelly invertebrates *Cambrotubulus* and *Anabarites sp.* pointing to a basal Nemakit-Daldynian age, while the first appearance of *Treptichnus pedum* around 70 m higher in the sequence coincides with the base of the Tommotian (*103*) that has been dated to 534 Ma (*104*). While age extrapolations on the basis of sediment thickness do not work in this setting because of its multiple erosion surfaces, its biota are remarkably similar to fossil remains that are considered a biostratigraphic marker in the middle Dengying Fm (Shibantan Member) in South China (*105, 106*), which has been dated to 551–541 Ma (*107*,*108*). Hence we tentatively assign the reported Khatyspyt sample to the 551–544 Ma age bracket. The Khatyspyt Fm contains exceptionally immature organic matter and high bitumen abundances (*105*), minimizing the danger of contamination. Furthermore, a recent report of relative sterane abundances in bitumen

from this unit has been corroborated by hydropyrolysis (*105*), which leaves little doubt about the syngenicity of this molecular signal.

19. Ara Group (18 samples): 547–539 Ma

The Ara Group comprises six transgressive carbonate-evaporite cycles (A1–A6), in addition to a basal carbonate unit (A0), that were deposited on a carbonate platform. During deposition of the A4 unit, a deeper subbasin opened and accumulated deep-water shales, as well as a silicalite unit. A negative carbon isotope excursion recorded during his interval is paralleled by the extinction of the sessile benthic metazoan *Cloudina* and was dated at 542 Ma (*109*). Multiple subsequent age dates were obtained for this sedimentary package (*55*), which constrained the middle of the A0 unit to 546.7 Ma and allowed the authors to deduce the average duration of a carbonate-evaporite cycle at 1–1.2 Myr for the A0–A3 period. Furthermore the age of the Ediacaran-Cambrian boundary was corrected to 541 Ma (base of A4 carbonate). Extrapolating this age upsection using the average duration of an A0–A3 carbonate-evaporite package, gives an age range of 547–539 Ma for the here reported samples from the Ara Group.

20. Siva oil (1 sample): ca. 550–530 Ma

Late Neoproterozoic to Terreneuvian (*110*) oils from the Siva Field in the Russian Urals (*111*) are characterized by a C_{29} sterane dominance that far exceeds any other sample in this study (*112*). While no quantification has been provided by Seifert and colleagues, a chromatogram (Fig. 13 in (*112*)) shows the near-absence of C_{27} steranes, thereby suggesting that the C_{27}/C_{29} ratio must have been exceptionally low, and certainly <0.05. To err on the cautious side, we have listed this sample with a C_{27}/C_{29} ratio of 0.1, which is still the lowest value amongst those reported in this publication. Given the poor age constraint, we have chosen a wide Late Neoproterozoic to Early Cambrian age bracket.

21. Zhetebay oil (1 sample): ca. 550–530 Ma ⭕

The Zhetebay oil was presented by Knoll and colleagues (*7*) as an example for one of a number of late Neoproterozoic/Terreneuvian petroleum systems, in which sterane signatures are dominated by the C_{29} homologue. Hence, we assign to it a broad age of 550–530 Ma.

22. Usolye Fm (7 sample): ca. 534–530 Ma ✖

The Usolye Fm was studied in cores from Kulindinskaya-1 well, which is located in the Katanga Saddle area of western Siberia. A Tommotian age has been assigned to this formation (*74*), placing it in the narrow 534–530 Ma age range.

23. Lena-Tunguska oils (1 sample): ca. 534–526 Ma ⭕

A strong predominance of C_{29} steranes has been observed in many late Neoproterozoic-Terreneuvian oils of the Lena-Tunguska region in Eastern Siberia, but not quantified (e.g. *113*). A similar observation on sterane distributions was made on oils from the same region by Summons and Powell (*114*). We reconstructed rough C_{27}:C_{28}:C_{29} sterane ratios on the basis of MS/MS chromatograms presented for one sample, using the maximum intensity (ion counts) on the mass transition traces *m/z* 372 to 217, 386 to 217 and 400 to 217 (*114*). Geochemical characteristics point to a hypersaline-carbonate source rock, implying a likely origin of these fluids from evaporites within the Tommotian Usolye Fm or the Atdabanian Belsk Fm (*74*). Hence we assign these oils a Terreneuvian age in the range 534–526 Ma (*104*).

24. Mount Cap Fm (1 sample): ca. 540–520 Ma ⭕

Oil shows in Cambrian siliciclastics encountered by drill holes in the Colville area of the Northwest Territories, Canada, have been attributed to a petroleum source level in the Terreneuvian Mount Cap Fm (*115*). This unit comprises both source (*116*) and reservoir

facies in the form of deltaic-marine siltstones and glauconitic sandstones, as well as shallow-water shales. A thin interval of laminated, dark brown organic-rich shale has been identified in the Mount Cap Fm at a depth of 943 m in drill hole Colville D-45 (*117*) with a TOC content of 9.5% and a Hydrogen Index of 768 (*116*). Analysis of extracted bitumen from this level has revealed a high abundance of saturated hydrocarbons, characterized by *'... a predominance of C_{29} over C_{27} steranes*' (*117*), although their ratio is not specified. We have assigned a broad Terreneuvian age bracket of ~540–520 Ma to this bitumen.

25. Diamond Bog Dolomite (1 sample): ca. 535–520 Ma

The Diamond Bog Dolomite is an Terreneuvian–Cambrian Series 3 unit (*118*, *119*) of the eastern Warburton Basin, South Australia, which overlies the Mooracoochie volcanics and unconformably underlies the Cambrian Epoch 3 Kalladeina Fm (*119*). One sample, recovered from drill hole Gidgealpa-7 was studied by Hallmann and colleagues (*120*) in the context of regional petroleum migration across the Cooper-Eromanga-Warburton Basins. A broad Terreneuvian to Cambrian Epoch 3 age is assigned on the basis of its stratigraphic relationship to the Terreneuvian Mooracoochie Volcanics and the <510 Ma Kalladeina Fm.

5. Samples with contaminated or 'empty' interior biomarker signatures

Five stratigraphic units were studied, of which none of the analyzed samples have yielded uncontaminated and indigenous steranes, or where the indigeneity of detected steranes could not be unambiguously verified (table S2). These samples are not used in arriving at the conclusions of the present study and hence they will not be described in detail.

6. Timing and verification of the post-Marinoan C_{27} / C_{29} sterane dichotomy

The most important time slice for the interpretation of our results is situated directly after the Marinoan deglaciation. Whilst the pre-Cryogenian world is demonstratively

dominated by C_{27} steroid biosynthesis in eukaryotes—although we currently cannot exclude that this represents a bacterial signal (*4*)—the immediate post-Marinoan exhibits a strong steroidal gradient before transitioning into an Earth system where global steroid metabolism is dominated by C_{29} homologues, thereby testifying to the ecological success of the probably green algal host organism that acquired this trait. While in Figure 1b we use the rather wide time bracket of 635–600 Ma, three of the studied settings likely record a snapshot of the first <5 Myr after the Marinoan deglaciation. Bitumen in the Mirassol'Oeste Fm contains solely C_{27} steranes (i.e. still carries the pre-Marinoan signal) at a stratigraphic range ca. 15–31 m above the diamictite (although we should point out that one pyrolysate of this formation contained traces of C_{29} steranes—see fig. S5 and discussion further below). The Koniakari Group sample, which is still strongly C_{27} sterane-dominated, but does contain low abundances of C_{29} steranes, belongs to the upper part of the Marinoan cap carbonate in Kayes, Mali and is situated 6.3 m above the Marinoan diamictite. Samples from the SOSB derive from different stratigraphic levels of the Masirah Bay Fm. All of these samples are already strongly dominated by C_{29} steranes. The oldest of these samples derives from drill hole Zafer-1, where the Masirah Bay Fm extends from 1962 to 2295 m depth and the Hadash cap carbonate from 2295 to 2305 m. The sample represents a composite of cuttings from 2280–2295 m interval. Given the amalgamated nature of this sample, we can only use the shallower depth (2280 m) in our interpretation and thus assume that this sample was deposited 25 m above the diamictite. Assuming a somewhat uniform carbonate production rate and accumulation speed, this means that the C_{27} dominated steroidal signature of the Mirassol'Oeste Fm is actually younger than the C_{29}-dominated signal in the Masirah Bay Fm, and definitely younger than the rocks from Mali, which contain both C_{27} and C_{29} steranes with a strong predominance of C_{27} homologues.

Two older samples with a C_{29} steroid dominated signature were reported from the SOSB by Love and colleagues (*83*), who interpreted the age of these rocks from the Fiq Member of the (upper) Ghadir Manquil Fm as being younger than the Sturtian diamictite yet older than the Marinoan cap carbonate. With improved age constraints (*22*) this would constrain the samples to 659–635 Ma. Yet given that (a) the samples studied by Love and colleagues are located only ca. 230 and 110 m below the Marinoan cap carbonate, despite

a much greater thickness of the Marinoan diamictite in this region (*55*), and (b) the stratigraphic profiles in Love et al. (*83*) reveal the absence of the Saqlah Member basalts (which forms the lowest part of the Ghadir Manquil Fm and separates the younger Fiq Member diamictites from older interglacial deposits), we conclude that the age of these two samples is Marinoan and that they were likely deposited during the deglaciation phase. A maximum age around 645–650 Ma (i.e. late interglacial) was suggested by Paul Hoffmann (personal communication on May 19th 2017): "*If the stratigraphy of the GM-1 and MQR-1 cores is anything like that on the Mirbat Coast, then Gordon's [viz. Love et al.: reference 83] lowermost sample in each core should predate the Marinoan glacial onset (ca. 645 Ma), and substantially postdate the Sturtian glacial termination (659 Ma). However, neither Fig. 1 nor fig. S1 of Love et al. 2008 allows us to say this with confidence*." In either case, the rise of C_{29} steroid biosynthesis can be firmly and confidently constrained to the Cryogenian and we can thus confidently exclude that the observed dichotomy (Fig. 1 b) actually represents a temporal transition, where C_{29} steroid biosynthesis emerged rapidly after the Marinoan deglaciation.

Another important point that needs to be considered in light of the observed dichotomy in steroid patterns after the Marinoan deglaciation is the originality of the fossil C_{27} *vs.* C_{29} steroidal signal, which is discussed below.

Mirassol d'Oeste Formation

Bitumen in the Mirassol d'Oeste Fm contains a C_{27}-only sterane signature, which persists between 15.2 m and 31.2 m above the base of the cap dolostone. Samples between 0 and 15.2 m are organically barren, while no samples higher than 31.2 m are in our possession. Given that bituminous nodules exist at a height of around 20 m in this unit, we must exclude the possibility that the observed C_{27} signature stems from a sub-Marinoan sourced, migrated petroleum fluid. All the available evidence argues against an allochthonous explanation for the signature. From a petrographic perspective, multiple stratigraphic layers contain very small, isolated bitumen spheroids, which were likely produced *in situ* and form due to the porous nature of the host rock, acting as both source and reservoir. Most importantly, in the Terconi area the diamictite rests directly on

crystalline basement rocks, implying that no regional source of older hydrocarbons exists. Detailed biomarker analyses of the Terconi section samples have revealed systematic changes in the distribution of steroid and hopanoid biomarkers, which would not exist if a migrated fluid had emplaced these compounds. Furthermore we observe systematic co-variation between biomarker hydrocarbons and metal abundances, in particular redox-sensitive authigenic lead concentrations (*60*). Given that the Pb is not associated with the organic matter, this again indicates that the observed relationship cannot be explained if the hydrocarbons had migrated from elsewhere. The same conclusion was reached in previous studies on hydrocarbons in the Araras Group (*121*,*122*). The most compelling argument for the syngenicity and non-migrated nature of the studied bitumens however comes from a hydropyrolysis analysis of isolated kerogen (Terconi quarry, 15.2 m). While the thermolysate of this pre-extracted kerogen did not contain any steroidal hydrocarbons (indicating the absence of any remnant free hydrocarbons), analysis of the subsequently generated pyrolysate revealed the presence of C_{27} steranes in exceptional relative abundance, the absence of C_{28} steranes as well as traces of C_{29} steranes (fig. S5).

Masirah Bay Fm

Initially we suspected that the persistent dominance of C_{29} steranes in *all* stratigraphic units of the SOSB (*75*) may be an artefact of oil staining. After all, the SOSB is a petroliferous depocenter that has recorded much petroleum fluid flow and records minimal variation in the pattern of major sedimentary steroids throughout the entire Ediacaran. This suspicion was highlighted by the inefficient extractability of the two Masirah Bay samples that we had analyzed in our laboratory: after multiple solvent extraction steps, every renewed extraction would still yield high levels of bitumen. Hydropyrolysis of SOSB rock, including those from the Masirah Bay Fm, had been used in the past as a means of verifying the indigeneity of the detected hydrocarbons (*83*). During this procedure, hydrocarbons covalently bound to the macromolecular kerogen structure are catalytically cracked: the resulting bitumen should thus represent certainly indigenous hydrocarbons. But if the samples are stained by an allochthonous oil phase that has pervaded the rock to a degree that it is nearly impossible to remove with organic solvents, these contaminants would also contribute to the hydropyrolysate.

To ascertain the indigeneity of the C_{29}-dominant sterane signal in the Masirah Bay Fm, we tested an excessive extraction approach prior to hydropyrolysis that far exceeded the intensity of extraction commonly needed in organic geochemical studies. Ultrasound-assisted solvent extractions with DCM were performed until the color of the resulting bitumen-solvent mixture faded to clear (only after 14 extractions with 30 mL DCM each). At this point we dried the rock powder and sequentially digested carbonate and silicate minerals with HCl_{aq} and HF_{aq}. Re-extraction of the resulting kerogen concentrate again yielded a deeply dark-colored extract. We again extracted these kerogens as described above, until the final yellow hue faded and extracts remained clear (again after 14 extractions with 30 mL DCM each). The last extract that still yielded detectable hydrocarbons still exhibited a C_{29}-steroidal dominance. After drying, the kerogen was treated with pyridine (this swells the structure, potentially enhancing solvent accessibility) and again re-extracted (with a pyridine-DCM 1:1 mixture) until no color was discernible (5 times with 10 mL each), prior to hydropyrolysis at the University of Nottingham (William Meredith and Colin Snape), which was preceded by a thermal desorption step as outlined in the Methods section. The absence of discernable hydrocarbons in the thermal desorption step confirmed that the sample was completely and exhaustively extracted. We should point out that this extraction procedure is absolutely excessive and not commonly needed. In this particular case we were absolutely certain that no oil or bitumen remained in the kerogen sample prior to hydropyrolysis, yet the pyrolysates were still characterized by the same elevated C_{29}/C_{27} sterane ratio. Hence, the originality and indigeneity of the C_{29} steroidal dominance in the oldest Masirah Bay samples has been unambiguously verified.

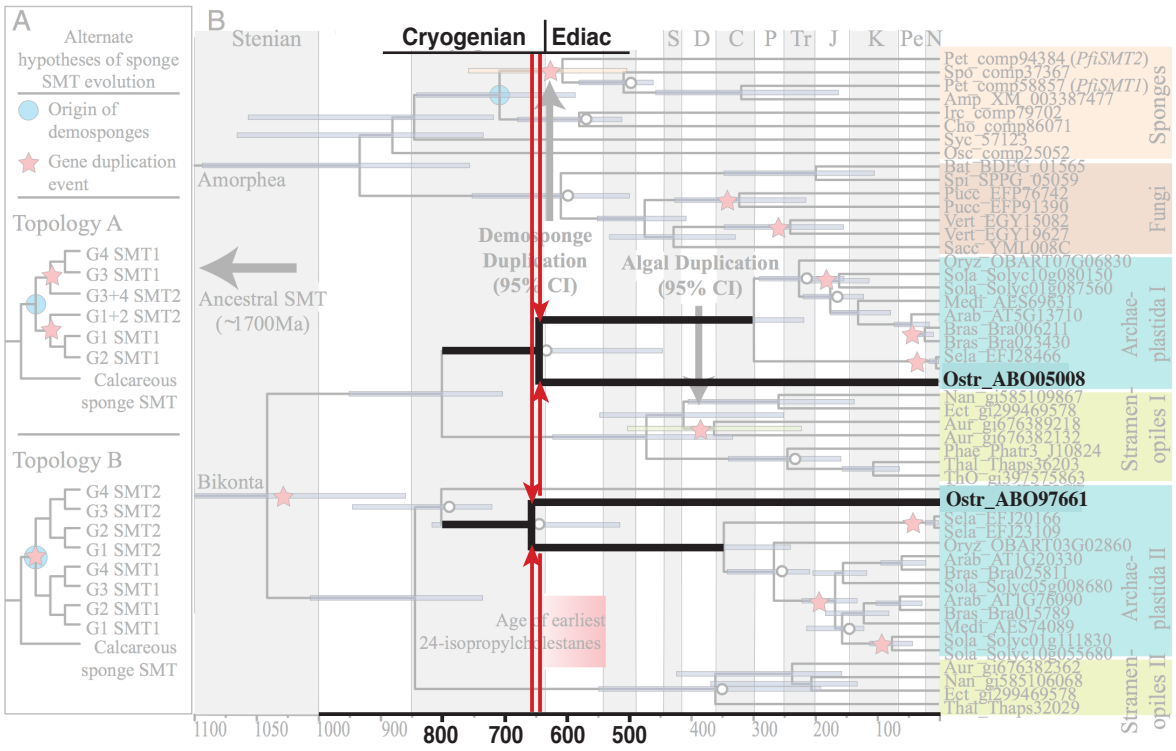

fig. S1. Molecular clock analysis of SMT genes (*30*) reveals a divergence of green algae during the Late Cryogenian. This time-calibrated phylogenetic tree of SMT genes was modified after Figure 3 in Gold et al. (*30*), highlighting the late Cryogenian divergence of the two SMT gene copies in the prasinophyte green alga *Ostreococcus*, and supporting a Snowball Earth origin for stigmasteroid (*1*) biosynthesis in green algae.

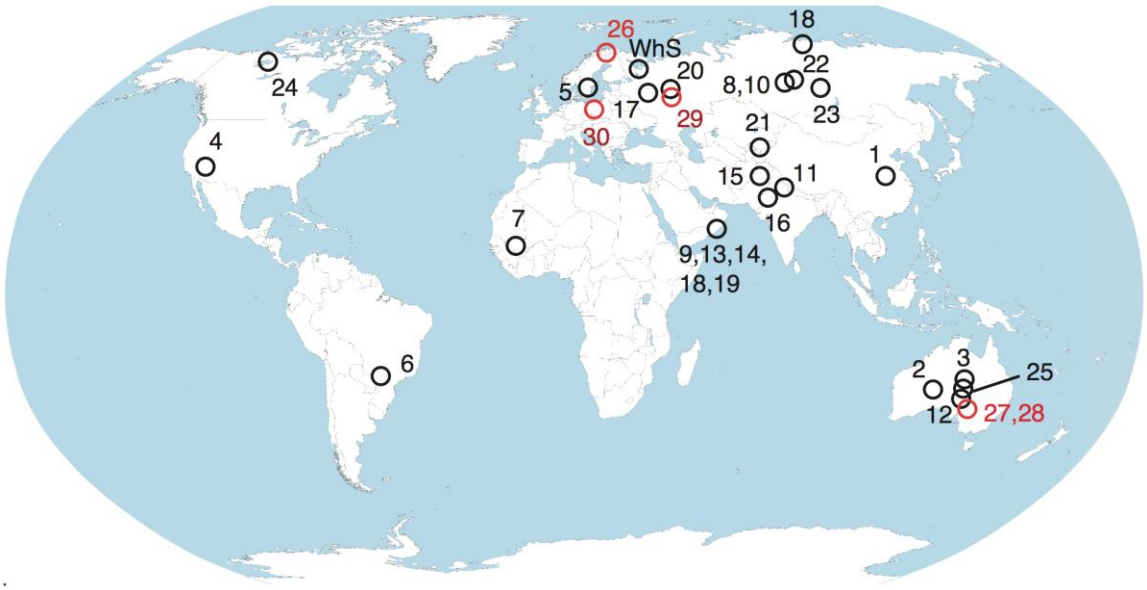

fig. S2. Studied sample locations on a modern continental configuration. Closed black circles indicate localities where at least one studied sample yielded indigenous, unambiguous and non-contaminated steroidal signatures. Red circles indicate localities where none of the studied samples yielded credibly indigenous steroidal signatures. Numbers correspond to those in Section 5 of the Supplementary Material, fig. S3 and in table S1.

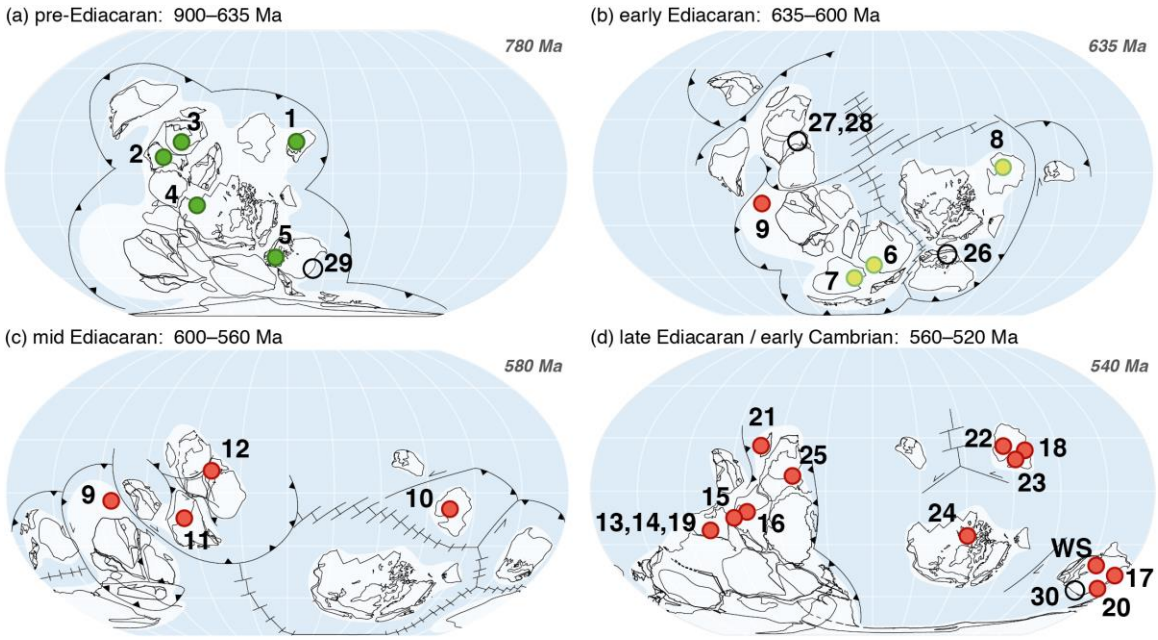

fig. S3. Studied sample locations in a palaeogeographic context after (*31*). This annotated version of Fig. 1 places samples discussed in Section 5 of the Supplementary Material and listed in table S1 in the context of palaeogeographic origin. Numbers correspond to those in fig. S2, table S1 and Section 4 of the Supplementary Material. The color code corresponds to that in Fig. 1. Open circles indicate locations with no indigenous steranes.

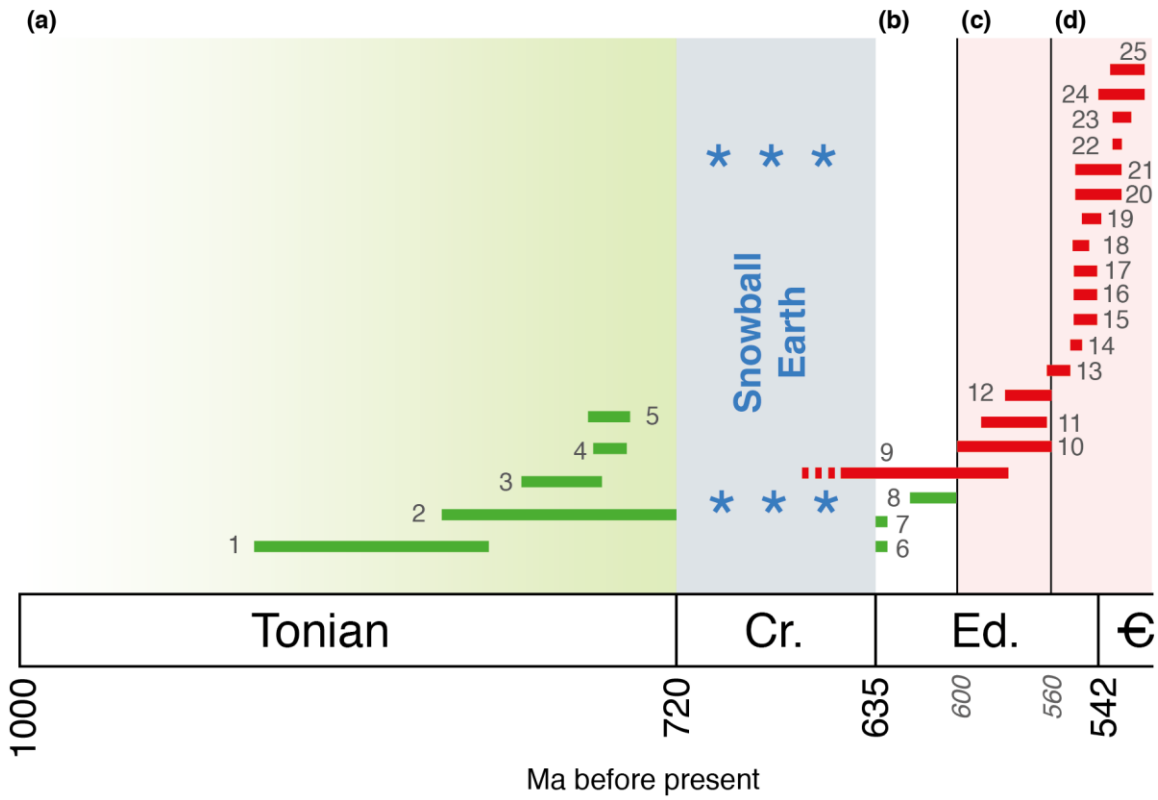

fig. S4. Estimated maximum time brackets of the studied samples. This schematic figure visually places samples described in Section 4 of the Supplementary Material and listed in table S1 into their relative temporal context. Numbers correspond to locations shown in fig. S2 and fig. S3, table S1 and Section 4 of the Supplementary Material. The four time brackets (**a**–**d**) correspond to those in Fig. 1. Green bars denote the sole presence or dominance of C_{27} steranes, while red bars denote the dominance of C_{29} steranes.

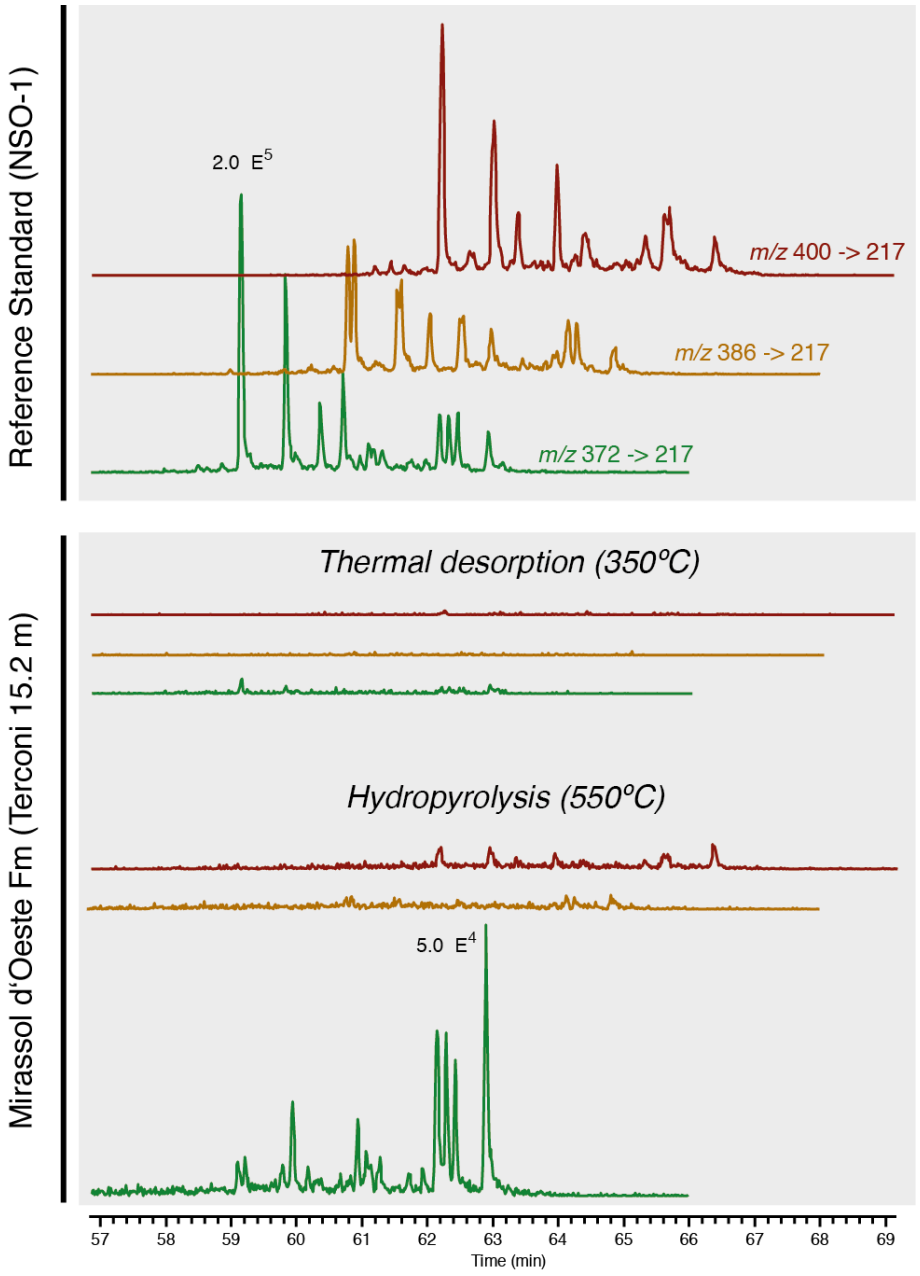

fig. S5. Hydropyrolysis of an earliest Ediacaran cap carbonate sample from the Mirassol d'Oeste Fm verifies the C_{27} sterane–dominated signature found in other samples from this unit and confirms their syngenicity to the host rock.

table S1. (see separate document: table S1) Steranes in Neoproterozoic rocks. Origin, age, depositional environment, lithology and sterane content of sedimentary rocks used in this study. Color codes correspond to those in Fig. 1.

table S2. Unsuitable samples. Rocks from the here listed sedimentary formations were studied but did not yield unambiguously indigenous steranes and were hence not used to come to the conclusions presented in this study.

#	Stratigraphic unit	Samples studied	Estimated age range (Ma)
26.	Nyborg Fm	3	~ 635
27.	Nuccaleena Fm	2	~ 635
28.	Brachina Fm	2	~ 635–630
29.	Min'yar Fm	15	900–800
30.	Lublin Fm	4	550–540