Possible Evolution of Mobile Animals in Association with Microbial Mats

1. Living in Hypoxic Conditions and Contending with H₂S
Bailey et al. explored the viability of oxygenic biomats as a source of local oxygen⁴. They surmised that anoxic conditions occur too closely to biomat levels to afford animals living space and that in darkness sulfidic conditions would be elevated to levels that would preclude animal colonization. Marine regions poor in oxygen are, in fact, characterised by the presence of strikingly few species, but are not necessarily devoid of invertebrate animals. In fact several species of annelids show resistance to hypoxic conditions over time periods as long as 2 weeks (e.g. Streblospio benedicti, Nereis spec, Capitomastus minimus and Capitella capitata)²,³,⁴. Importantly Steblosoma benedicti, has the ability to survive moderate concentrations of hydrogen sulfide (< 66 μM) for longer than 2 days⁵. Considering the ancient lineages represented by annelids, the low O₂ concentrations in the Ediacaran oceans, and recent work indicating sulfide gas as regulator of metabolic activity and inducer of suspended animation in some higher animals⁶, it is plausible that resistance to elevated sulfide conditions evolved early in metazoans.

2. Types of Ichnofossils Potentially Associated with Oxygen-Mining Lifestyles
Oxygen-exploitive trace fossils should display some of the following characteristics: (1) an open connection to the sediment-water interface with optimization of burrow surface area or volume beneath biomat zones; (2) situated at the base of biomats and has no connection to the sediment-water interface; or, (3) the trace fossil is preserved immediately beneath fossil biomats and can be shown to be backfilled during trace-fossil emplacement.

Trace fossils that maintained a connection to the sediment-water interface—but show optimization of burrow-volume / surface-area beneath biomats—are characterized by serial branching beneath the biomat (e.g., Chondrites Fig. 3A). A variation on branching forms may include sinusoidal to spiral forms, which act to optimize surface area within a sediment volume (e.g., Gordia, Helminthoidichnites, Helminthopsis, Helminthorhaphe-Spiroraphe, Treptichnus). In these and similar examples, the trace maker optimized its surface area adjacent to the biomat to maximize oxygen diffusion into the burrow (Figs 2B and 3B). Burrows that occur below biomats and were not connected to the sediment-water interface are difficult to recognize due to limitations of preservation (e.g., Nenoxites, Torrowangea). However, examples of the third scenario are generally manifested as straight through meandering backfilled burrows observed at
the base of biomats (Figs 2C and 3C-D). These occurrences are significant because the animal’s only potential connection to the sediment-water interface (the burrow) was filled behind the animal as it proceeded beneath the biomat, eliminating its ability to irrigate the living space with oxygenated water. In other words, animals living in back-filled burrows positioned below biomats must have received their oxygen from the biomat itself.

If undermat oxygen-mining behaviours can be applied to the Ediacaran record then their behaviors would be largely confined to undermat and within-mat probes or meanderings (e.g. *Oldhamia* or *Helminthoidichities*), or—later in the Ediacaran—burrows that show below-mat spatial optimization but retained connections to the sediment-water interface (e.g. *Treptichnus* isp. ⁷). It is important to note that the previous interpretations of these traces (as grazing or deposit-feeding traces, or as animal domiciles) are viable and that local oxygen availability could merely have enabled the exploitation of food resources and living space.

3. Preservation of Ediacaran Bed Surfaces
Spatio-temporal variations in biomat-associated oxygen helps to explain the surficial pyritization that is ubiquitous on well-preserved Ediacaran bed surfaces. And it reinforces hypotheses that this pyritization reflects mat-mediated oxygen starvation of subsurface sediment⁶,⁹, whereby biomat degradation following sudden burial, via bacterial sulfate reduction, produces a pyrite "death mask" that allows the moulds of sessile Ediacaran organisms to be preserved. Another process might also explain the occurrence of “death masks”, which is that the sessile animals preferentially colonized sulfide-oxidizing biomats when and where oxygen-concentrations in the water were not dependable leading to the preservation of pyritized vendobionts.

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**Taxonomic Appendix**

*Eochondrites rectangulus*, new ichnogenus and ichnospecies

Seilacher, 1999¹: Biomat-related lifestyles, Fig. 12;
Seilacher, 2007², plate 49

*Locus typicus*: Upper Cambrian of the Huqf area (Oman)
Type = Cast of imaged sample held at Tubingen.

Diagnosis: Bed-parallel burrow systems consisting of a straight base tunnel (variable diameter 2-7 mm, several decimeters long and retrusively backfilled shorter probes that branch off at right angles on one or both sides. The primary tunnel may also branch, but characteristically at angles of 30 - 40°.

Interpretation: The animal that made *Eochondrites* did so by digging a straight base tunnel 2-3 mm in diameter and up to 20 cm long. Then it produced secondary probes on either side. The probes vary in length, but have been observed up to 15 cm long, and meet at the base tunnel at right angles (except in terminal positions). The probings expand in width towards the tip. We interpret *Eochondrites* as an undermat miner that browsed the decaying zone of the mat. The cohesive nature of the sub-mat sediment and the overlaying mat permitted the excavation of enlarged and irregular chambers that would not be possible in normal sedimentary softgrounds. The probings also served for pumping O₂ from the active mat on top. As such the probes are equally spaced and sequentially backfilled to ensure pumping integrity from the active
oxygenated conduit. The absence of a clear connection to the sediment-water interface and the presence of continuous and progressive backfilling suggest that for *Eochondrites* the only source of dissolved oxygen would have been from the oxygen mat itself.

Figure S1. Location map of the Los Roques Archipelago. Inset are details of Augustin and Pirata islands, where the studied lagoons are located (lagoons in light grey).
Figure S2. Example of the colonized biomat from Augustina (A) and Pirata (B) lagoons. The bubble texture in (B) is the result of within-mat O2 production. C. Close-up image of an in situ animal (Diptera: Ephydridae) living near the base of the biomat. The animal is extending itself in efforts to escape the mat, following exposure.
Figure S3. Clark-type oxygen microelectrodes were used to measure the nighttime *in situ* oxygen and hydrogen sulfide partial pressures in the bottom-water, biomat and underlying sediment. Shown here are 2 night profiles of dissolved oxygen and hydrogen sulfide in the water above and within the biolaminated sediments at Pirata Lagoon. In both examples, O$_2$ concentrations within the water are lower (2 to 8 times) than the O$_2$ concentrations during the day. Within the biomat, O$_2$ is essentially absent. At night H$_2$S is present nearly to the biomat-water interface, whereas it is not detectable at the biomat’s base during the day (shown in Figure 1).
Figure S4. Biomat dwellers. A. Diptera: Ephydridae; B. Diptera: Stratiomyidae; C. Coleoptera: Hydrophilidae; D. Diptera: Tabanidae; and one free swimmer (E) Heteroptera: Corixidae
Figure S5. Laminated (unburrowed) part of lagoonal sediment where the biomat was poorly developed. No macrofauna were observed in this (and other) core. The black arrows show where the lamination is well preserved. The upper part of the core is green (suboxic zone), the top of which resided at the sediment-water interface. Due to the dark color of sediment while it sits in the suboxic zone, lamination is difficult to discern until it passes below that level (tan-coloured sediment).
Figure S6. A and B. Photograph and schematic of the trace fossil *Eochondrites rectangulus*. Schematic emphasizes the branching morphology and cavernous excavations at branch termini. C. Schematic interpretation of *E. rectangulus* construction.