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Ezaz-Nikpay, A., Wedlake, G.O., Poulton, S. et al. (Accepted: 2026) Microfossils from Bitter Springs mudstones evidence the impact of lithology and depositional environment on Tonian microbiota diversity and preservation. Palaios. ISSN: 0883-1351 (In Press)

MICROFOSSILS FROM BITTER SPRINGS MUDSTONES EVIDENCE THE IMPACT OF LITHOLOGY AND DEPOSITIONAL ENVIRONMENT ON TONIAN MICROBIOTA DIVERSITY AND PRESERVATION

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ABSTRACT

Proterozoic organic-walled microfossils are preserved in a variety of lithologies worldwide. However, the effect of lithology and depositional environment on the taxonomic composition and preservation quality of microfossils within a single geological succession has only been well-studied for a few deposits. Here, we document microfossils in mudstones from the ~800 Ma Bitter Springs Group, Australia, and compare these to the well-studied chert-hosted microfossil biota in the Loves Creek Formation. Mudstones from the Loves Creek Formation yielded no fossils, whereas nine established Proterozoic taxa were recovered from Johnnys Creek mudstones, with some preserving features such as medial splits, intracellular inclusions, and multicellularity.

Comparison of microfossil assemblages with previously published Fe-speciation data for the same sample horizons reveals that the only eukaryotic taxon we report, *Cerebrosphaera*, occurs in a mudstone deposited under oxic conditions in the Johnnys Creek Formation, possibly reflecting the ecological preference of eukaryotes. The diversity of microfossils in Johnnys Creek mudstones is lower than in Loves Creek cherts. Microfossil preservation quality is also higher in the cherts, including three-dimensional preservation, with some cells displaying intricate intracellular features. This comparison between the biotas of the Bitter Springs mudstones and cherts shows how both taxonomic composition and preservation quality may vary between different lithologies and depositional environments from within the same geological succession. Thus, the distribution of lithofacies in time and space across the Proterozoic may bias the early fossil record.

INTRODUCTION

The early evolution of microbial life can be traced across the Proterozoic Eon (2,500–539 million years ago, Ma) using the record of organic-walled microfossils (e.g., Anderson et al., 2024). For example, these microfossils document the first appearance of microbial eukaryotes 1,780–1,730 Ma (Javaux, 2025), as well as organisms with simple multicellularity in rocks older than 1,630 Ma (Miao et al., 2024), and red and green photosynthetic algae at ~1,000 Ma (Butterfield, 2000; Tang et al., 2020). Organic-walled microfossils have also been used to track biodiversity across the Proterozoic (Tang et al., 2024; Porter et al., 2025) and to indicate how this diversity was partitioned between depositional environments, providing insight into the variability of Proterozoic coastal ecosystems (Knoll et al., 1991; Buick and Knoll, 1999; Muscente et al., 2015; Javaux and Knoll, 2017).

However, the fossil record of early life not only captures biological evolution (Porter and Riedman, 2023; Anderson et al., 2024; Tang et al., 2024), but is also a product of preservational bias imposed by sedimentary setting, with organic-walled microfossils being preserved across a range of sedimentary lithologies (Porter et al., 2025) that represent distinct depositional environments and taphonomic pathways. The foremost of these microfossil-bearing lithologies are early diagenetic cherts and mudstones/shales (together, they account for almost all eukaryote-bearing assemblages older than ~700 Ma; Porter et al., 2025). Early diagenetic cherts were typically associated with shallow marine, most commonly peritidal, environments (Knoll, 1985; Maliva et al., 1989; Maliva et al., 2005; Manning-Berg and Kah, 2017). In such environments, evaporation within silica-rich Proterozoic waters promoted high silica saturation (Maliva et al., 2005), and continental run-off likely enhanced near-shore productivity, leading to abundant nuclei for chert precipitation (Leo and Barghoorn, 1976; Knoll, 1985). Microbial mats and benthic communities were preserved in situ, with silica precipitation often inhibiting their decay and compaction (Manning-Berg et al., 2019). Proterozoic cherts can yield exceptional three-dimensionally preserved microfossils that retain delicate cellular structures and intracellular features (e.g., Schopf, 1968). However, the nearshore restriction of this fossilization mode means that chert-hosted assemblages may record only a narrow environmental subset of Proterozoic ecosystems.

By contrast, fossiliferous Proterozoic mudstones were deposited across a broader range of marine and marginal marine environments, from shallow shelf settings to deeper basinal environments (e.g., Javaux and Knoll, 2017). This wider environmental distribution means that mudstones have the potential to capture a more ecologically diverse range of microbial organisms as organic-walled microfossils, including both benthic and planktonic taxa. However, the taphonomic pathway in mudstones differs fundamentally from that in cherts. Organic-walled microfossils preserved in mudstones are typically two-dimensional,

reflecting compaction during burial. Furthermore, transport through the water column prior to burial may expose organic matter to oxidative degradation, reducing preservation quality (Butterfield, 1995). The internal contents of cells, often biologically informative features, are commonly lost to decay (Carlisle et al., 2021). The composition of mudstones, specifically their total organic carbon (TOC) content and clay mineral assemblages, can account for some variation in the quality of their preserved microfossils (Anderson et al., 2020; Woltz et al., 2021; Woltz et al., 2023).

Since microbial organisms are commonly distinguished from one another based on diagnostic cellular level features, retaining enough fidelity to resolve such features is vital to the accurate interpretation of microfossils (e.g., Anderson et al., 2024). For example, microfossils preserving plated cell walls have been used to evidence the evolution of intracellular trafficking and exocytosis (Knoll et al., 2006; Javaux and Knoll, 2017; Riedman et al., 2023), and concentric ridges preserved on the vesicle walls of certain taxa have been used to argue for the emergence of programmed encystment phases (Pang et al., 2013; Pang et al., 2015). In settings where microfossils are not preserved with enough fidelity, diversity may be underestimated or key biological features missed. Thus, given the temporal distribution of fossil assemblages in cherts and mudstones globally (e.g., Porter et al., 2025), effective reconstruction of the early evolutionary history of microbial life requires an understanding of the potential influences or biases that host lithology can exert on the composition of preserved biotas and the fidelity with which morphological features are retained.

Few studies have directly compared the diversity, taxonomic composition and preservational quality of microfossil assemblages from different lithologies that represent distinct depositional environments within a single geological succession. Such comparisons are essential for distinguishing ecological signals from preservational bias, and for

98 understanding how lithological variability may influence our interpretation of Proterozoic
99 life. Previous work, for example on the Ediacaran Doushantuo Formation, has demonstrated
100 that contrasting lithologies (in that case cherts and phosphorites) can significantly affect both
101 fossil recovery methods and preservation quality, impacting observed diversity (Liu et al.,
102 2014; Xiao et al., 2014), but detailed comparisons between the two most common
103 repositories—cherts and mudstones—remain limited.

104 The Tonian Bitter Springs Group of central Australia (Fig.1A–D) offers such an
105 opportunity to compare the diversity and taphonomy of chert and mudstone hosted biotas. It
106 hosts one of the most well-preserved chert biotas of the Proterozoic, first documented in the
107 1960s (Barghoorn and Schopf, 1965; Schopf, 1968). Early diagenetic peri-tidal cherts from
108 the Loves Creek Formation at the Ross River locality (see Fig. 1B) are reported to have
109 yielded over 24 genera comprising 30 species of microfossil taxa (Schopf, 1968; Schopf and
110 Blacic, 1971). Cherts from the stratigraphically-equivalent Ellery Creek locality (see Fig. 1B)
111 yielded a similar reported microfossil diversity (Barghoorn and Schopf, 1965; Schopf and
112 Blacic, 1971; Knoll and Golubic, 1979). Accompanying mudstone strata from the Gillen and
113 Loves Creek formations have been examined for microfossils by Zang and Walter (1992).
114 However, the techniques used to recover the microfossils in that study involved potentially
115 destructive processes such as shaking, possibly artificially masking their preserved diversity.
116 Re-evaluation of the material presented in that study also suggests that some of the reported
117 microfossils may be pseudofossils (see Supplementary Table 1). Consequently, re-
118 examination of Bitter Springs mudstone strata using gentle, low-manipulation microfossil
119 extraction techniques (e.g., Butterfield and Harvey, 2012) has the potential to provide an
120 enhanced dataset to undertake new comparisons of diversity and preservation quality between
121 chert and mudstone hosted biotas from the same broad geological succession.

Here, we report new microfossils (Figs. 1G, 2–4) from mudstones of the unstudied Johnnys Creek Formation of the Bitter Springs Group. We additionally investigated mudstone samples from the Loves Creek Formation, but here we recovered no microfossils (Fig. 1G). We compare the diversity and preservation quality of the Johnnys Creek microfossils recovered in our study to the previously reported mudstone biotas from the Gillen and Loves Creek formations, as well as the famous chert biota from the Loves Creek Formation. To enable accurate comparisons, we re-evaluate the diversity reported in previous studies of the Bitter Springs Group in light of subsequent taxonomic research on organic-walled microfossils (see Supplementary Table 1).

GEOLOGICAL SETTING

The Tonian Bitter Springs Group

Overview.—The Bitter Springs Group forms part of the northern Amadeus Basin sedimentary succession in central Australia (Fig. 1A, 1B), occurring between the Heavitree Quartz and Areyonga Formation (Fig. 1D) (Swanson-Hysell et al., 2012). It comprises >1,500 m of shallow marine carbonates, with interbedded mudstones and evaporites (Fig. 1D) (Swanson-Hysell et al., 2012; Klaebe et al., 2017). The Amadeus Basin has been subjected to only very low-grade metamorphism (Hand and Sandiford, 1999), allowing for high quality preservation of microfossils (Schopf, 1968; Schopf and Blacic, 1971; Schopf et al., 2005; Schopf and Kudryavtsev, 2009). A substantial population and diversity of microfossils have been reported in early diagenetic cherts from carbonate facies of the Loves Creek Formation (Barghoorn and Schopf, 1965; Schopf, 1968; Schopf and Blacic, 1971; Knoll and Golubic, 1979). Microfossils have also been recovered from mudstones of the

145 Gillen and Loves Creek formations (Zang and Walter, 1992), but the Johnnys Creek
146 Formation has not previously been studied.

147 *Paleoenvironmental setting.*—Klaebe et al. (2017) and Hill et al. (2000) describe the
148 paleoenvironmental evolution of the Bitter Springs Group. Sediments of the basal Gillen
149 Formation (Fig. 1D) record fluctuations between marine and lacustrine conditions, indicating
150 periods of relative sea level rise and fall, with some evidence for restriction (e.g., bedded
151 evaporites). The overlying Loves Creek Formation begins with a general marine
152 transgression, followed by an ~200 m thick cyclic stromatolite facies (Fig. 1D, 1E).
153 Desiccation cracks and red beds record shallower subaerial environments within this facies,
154 and precipitated gypsum and anhydrite from concentrated brines suggest increasingly
155 evaporitic conditions. This sequence is followed by interbedded siltstones and carbonates
156 towards the top of the Loves Creek Formation, indicating a relative sea level rise. The
157 uppermost portion of the succession, the Johnnys Creek Formation, records ~500 m of
158 persistent red-bed silts and mudstones (Fig. 1D, 1E), indicative of a prolonged period of low
159 sea level with salt flats and shallow ponds. In many locations in the Amadeus Basin,
160 subglacial erosion at the top of the Bitter Springs Group has led to the loss of the uppermost
161 beds of the Johnnys Creek Formation.

162 *Age constraints.*—The maximum age of the Bitter Springs Group has been
163 constrained to between 1,068 ± 2 and 1,085 ± 2 Ma by $^{40}Ar/^{39}Ar$ radiometric dating of biotite
164 grains from country-rocks in contact with underlying dykes in the southern Musgrave and
165 northern Arunta Paleoproterozoic basement blocks (Schmidt et al., 2006). An estimate of its
166 minimum age is provided by overlying glacial deposits of the Areyonga Formation, which are
167 suggested to be equivalent to the global ~720–660 Ma Sturtian Snowball Earth glaciation
168 (Swanson-Hysell et al., 2012). $^{187}Re/^{187}Os$ radiometric dating of black shales from the Aralka

169 Formation (~150 m up section from the Bitter Springs Group), provides a hard minimum age
170 of 657.2 ± 5.4 Ma (Kendall et al., 2006) for Bitter Springs deposition.

171 The majority of mudstone horizons examined for microfossils in this study are
172 additionally constrained by carbon isotope chemostratigraphy (non-fossiliferous samples W7
173 and W8 sit stratigraphically below this constraint). These horizons sit above a negative
174 carbon isotope excursion, the Bitter Springs Anomaly (Fig. 1F) in the Loves Creek
175 Formation, which has been documented worldwide (e.g., Canada, Svalbard and Ethiopia;
176 Halverson, 2006; Halverson et al., 2007; Swanson-Hysell et al., 2015), and records a shift in
177 the $\delta^{13}C$ composition of carbonate rocks from around +5‰ to -4‰ (Swanson-Hysell et al.,
178 2012). A $^{206}Pb/^{238}U$ radiometric age from zircons within a tuff unit of the Fifteenmile Group,
179 Yukon, Canada, constrains the onset of the Bitter Springs Anomaly to 811.51 ± 0.25 Ma
180 (Macdonald et al., 2010), and its termination is constrained by a $^{206}Pb/^{238}U$ radiometric age
181 of 788.72 ± 0.24 Ma from zircons within a tuff horizon in the Tsedia Formation, Ethiopia
182 (Swanson-Hysell et al., 2015). A Re-Os age of 799.6 ± 3.5 Ma from within the Bitter Springs
183 Anomaly in the Wynniatt Formation, northwest Canada, provides an additional constraint
184 (Shipman et al., 2026). The Bitter Springs Anomaly thus constrains the maximum age of
185 microfossils found in our Wallara-1 drill core samples (W32–49, see Fig. 1F, 1G) to <788.72
186 ± 0.24 Ma.

187

188 **METHODS**

189 Preparation of samples

190 A total of 19 mudstone samples were processed from the Loves Creek and Johnnys Creek
191 formations of the Bitter Springs Group (Wallara-1 drill core stored at the Northern Territory
192 Geological Survey, Alice Springs, see Fig. 1G and Supplementary Table 2) (24°36’55”S;

132°20’23”E) (Klaebe et al., 2017) to extract microfossils. Samples were selected based on total organic carbon (TOC) contents of <0.5 wt% (see supplementary information of Guilbaud et al., 2015), a known indicator for favorable preservation of organic walled microfossils in Proterozoic mudstones (Woltz et al., 2021; Woltz et al., 2023). Microfossils were extracted at the Department of Earth Sciences, University of Oxford, following the modified low manipulation protocol of Butterfield and Harvey (2012). Mudstone sample chips, 1–2 cm in size, were washed with deionized water to remove external contaminants and immersed for 48–72 h in 48% hydrofluoric acid to dissolve silicate minerals. Resultant acid-insoluble residues were passed through repeated baths of deionized water until a circumneutral pH was achieved. The residues were then filtered through a 45 μm brass sieve, with the >45 μm fraction retained for analysis. This process has previously led to the recovery of fossils with exceptional levels of detail (e.g., Butterfield et al., 1994; Butterfield, 2005).

Analysis of microfossils

Small volumes of each >45 μm fraction were subsampled in a petri dish and then examined using a Microtec HM-3 stereomicroscope on a Microtec HM-3 L4N incident and transmitted LED stand at the Department of Earth Sciences, University of Oxford. Potential microfossils were picked using a 20 μl pipette, then deposited on a glass slide and left to dry. Microfossils were examined and imaged using a Zeiss Axioscope A1 with a Zeiss Axiocam 212 camera at the Museum of Natural History, University of Oxford. All microfossils are deposited in the Museum of Natural History, University of Oxford.

Re-evaluation of species richness in previous studies of the Bitter Springs Group

Assignments of organic-walled microfossils presented in previous studies of the Bitter Springs Group (see Schopf, 1968; Schopf and Blacic, 1971; Zang and Walter, 1992) were re-

evaluated in light of subsequent taxonomic research to enable robust comparison to the microfossils reported in this study. Previous taxonomic reassessments (e.g., Riedman and Sadler, 2018; Porter et al., 2025) were used as a basis, expanding these to include prokaryotic material and combining them with critical review of formal synonymies (e.g., Butterfield et al., 1994; Sergeev et al., 2012). Where no previous reassessments or formal synonymies were available for a reported taxon, or where reported names have fallen out of general use, material was either informally synonymized (based on review of original diagnoses and figured specimens), or accepted without amendment in cases where either diagnosis and/or figured specimens do not provide enough information to confidently synonymize or reject assignments. Where figured specimens were too poorly preserved to assess, we omit these taxa from our updated assignments and estimates of species richness. We provide an overview of the updated assignments of our re-evaluation and updated diversity estimates for each study in Supplementary Table 1, alongside a list of taxa recovered in this study from the Johnnys Creek Formation. Note that our species richness estimates combine species within *Leiosphaeridia* and *Siphonophycus* to open 'spp.' nomenclature, to better compare diversity with fossils we recovered in this study, which uses the same nomenclatural convention.

RESULTS

Twelve mudstone samples from the Johnnys Creek Formation yielded organic-walled microfossils, with eleven of these occurring in the upper 200 m of the formation (Fig. 1G, Supplementary Table 2). Samples W42 and W47 contained the most diverse suite of microfossils (four taxa identified in each sample), with these samples being dominated by likely cyanobacterial filamentous taxa (e.g., *Oscillatoriopsis princeps*, ?*Pseudodendron anteridium* and *Siphonophycus* spp. in W42, and *Siphonophycus* spp. in W47). However,

neither of the samples examined from the Loves Creek Formation (W7 and W8, see Fig. 1G) yielded any microfossils.

In total across the Johnnys Creek Formation, nine taxa are reported: *Cerebrosphaera globosa* (Fig. 3A, 3B), *Glenobtrydion aenigmatis* (Fig. 4A–C), *Leiosphaeridia* spp. (Fig. 3C–J), *Oscillatoriopsis princeps* (Fig. 2A, 2B), *Palaeolyngbya catenata* (Fig. 2C–G), ?*Pseudodendron anteridium* (Fig. 2H), *Siphonophycus* spp. (Fig. 2I–K), ?*Symplassosphaeridium* sp. (Fig. 4J, 4K), *Synsphaeridium* sp. (Fig. 4D–I). Some poorly preserved microfossils are only tentatively assigned, denoted by a question mark. These taxa include one genus that has previously been unambiguously assigned to eukaryotes: *Cerebrosphaera* (Cornet et al., 2019). However, *C. globosa* is only represented by fragments, with no complete specimens recovered.

Medial splits, present in both individual *Leiosphaeridia* spp. specimens (n=3) (e.g., Fig. 3E) and occasionally in cells within clusters of *Synsphaeridium* sp. (n=2) (e.g., Fig. 4F), could be evidence for programmed encystment (Miao et al., 2019; Porter, 2020; Porter and Riedman, 2023), but a taphonomic origin for the splits through specimen crushing is impossible to rule out. Intracellular inclusions (ICIs), likely also taphonomic in origin (Carlisle et al., 2021), are preserved in five *Glenobotrydion aenigmatis*, seven *Leiosphaeridia* spp., and two *Synsphaeridium* sp. specimens (e.g., Figs. 3C, 3H, 4A–C, 4G). Evidence of possible multicellularity is present in both the clustered forms (e.g., *Glenobotrydion aenigmatis, Synsphaeridium* sp., ?*Symplassosphaeridium* sp.) and filamentous forms (e.g., *Oscillatoriopsis princeps*, *Palaeolyngbya catenata*). Clustered forms are composed of multiple spheroidal vesicles which do not exhibit any preferential arrangement or alignment, suggesting a lack of rigid developmental control. *Glenobotrydion aenigmatis* specimens also display gaps between constituent cells (e.g., Fig. 4A), while *Synsphaeridium* sp. and ?*Symplassosphaeridium* sp. specimens are more closely packed, with most cells overlapping

each other (e.g., Fig. 4D, 4J). In the filamentous forms *Oscillatoriopsis princeps* (see Fig. 2A, 2B) and *Palaeolyngbya catenata* (Fig. 2C–G), cells are arranged uniseriately into an unbranched trichome. In *P. catenata*, cells are also enveloped by an outer sheath and are frequently shrunken and disarticulated within this sheath (e.g., Fig. 2G). All of these cases are likely examples of simple multicellularity (or possibly of adventitious clustering in the case of taxa like *Synsphaeridium*), where there is no communication between the individual cells (Knoll, 2011). Lastly, a fragment of *Leiosphaeridia* in sample W36 shows small (<1 μm diameter), rounded openings on its surface (Fig. 3G). In other microfossils, e.g., *Cerebrosphaera buickii* (=*Cerebrosphaera globosa*, see Porter and Riedman (2016) for discussion of nomenclature) from the Tonian Svanbergfjellet Formation, Svalbard, perforations have been linked to predation or to the effects of degradative bacteria (Butterfield et al., 1994), and naked amoebae are known to perforate other organisms to access their intracellular contents (Berney et al., 2013; Porter, 2016). However, another plausible option is that the openings are a result of physical/chemical perforation (e.g., post-mortem pitting or abrasion, chemical alteration or mineral growth; Grey and Willman, 2009).

Species richness was overestimated in previous studies of the Bitter Springs Group (see Schopf, 1968; Schopf and Blacic, 1971; Zang and Walter, 1992), with many of the reported taxa being either insufficiently distinct from one another to warrant formal taxonomic separation or being misidentified on the basis of inconclusive morphological evidence. Our re-evaluation of previous microfossil reports (see Supplementary Table 1) identifies 16 species from mudstones of the Gillen Formation and only 2 species from mudstones of the Loves Creek Formation. A higher diversity of 25 species was identified in the accompanying cherts of the Loves Creek Formation, with many of these taxa (e.g., *Gloeodiniopsis*, *Oscillatoriopsis*, *Siphonophycus*) being common to both the Ross River (19 species identified) and Ellery Creek (22 species identified) localities.

DISCUSSION

Lithologies and associated depositional environments play central roles in controlling both the diversity and preservation quality of microfossils in the Bitter Springs Group. Comparing our results to previous work on Bitter Springs Group mudstones (Zang and Walter, 1992) reveals stratigraphic variability in diversity within the Bitter Springs Group and across the different lithofacies. Mudstones of the Gillen Formation preserved sixteen taxa, contrasting with those of the overlying Loves Creek Formation which preserved only two taxa (*Leiosphaeridia* spp. and *Siphonophycus* spp.) (see Supplementary Table 1; Zang and Walter, 1992). Whereas Loves Creek mudstones examined in this study were found to be barren, those studied from the Johnnys Creek Formation preserve an intermediate diversity of nine taxa, including many of the simple spheroidal forms (e.g., *Leiosphaeridia*), aggregates of spheroids (e.g., *Synsphaeridium*), and simple filamentous forms (e.g., *Siphonophycus*) common to the Gillen Formation assemblage. Bitter Springs mudstones also host possible eukaryotes, with *Comasphaeridium pollostum*, *Navifusa* sp. and *Simia annulare* previously reported from the Gillen Formation (see Supplementary Table 1; Zang and Walter, 1992), and fragments of *Cerebrosphaera globosa* reported in this study from the Johnnys Creek Formation.

Placing the new Johnnys Creek biota in the context of previously reported Fe-speciation data (Guilbaud et al., 2015) suggests that oxic (mean species richness = 2.3 ± 0.6) and equivocal (mean species richness = 2.1 ± 1.6) mudstone samples yield marginally greater microfossil diversity than ferruginous samples (mean species richness = 1.5 ± 1.9) (Fig. 5). Furthermore, the eukaryote *Cerebrosphaera globosa* is only found in a single oxic sample (W49) and a sample without accompanying Fe-speciation data, with equivocal and ferruginous samples dominated by likely prokaryotes. This taxonomic contrast between

Johnnys Creek mudstone samples might reflect the habitat preference of eukaryotes for oxic settings (e.g., Lechte et al., 2026). However, caution should be applied to these inferences given the limited number of samples in which microfossil diversity could be combined with Fe-speciation data (13 samples with Fe-speciation data, 9 of which yielded fossils).

By contrast, the previously reported chert-hosted biota of the Loves Creek Formation records a higher diversity of around twenty-five taxa, when combining the diversity from each locality (see Supplementary Table 1; Schopf, 1968; Schopf and Blacic, 1971). Of the nine taxa we report here from the Johnnys Creek Formation, only four (*Glenobotrydion aenigmatis*, *Oscillatoriopsis princeps*, *Palaeolyngbya catenata*, and *Siphonophycus* spp.) have also been reported in cherts from the Loves Creek Formation (although the genera *Oscillatoriopsis* and *Palaeolyngbya* are represented by different species in the cherts) (see Supplementary Table 1; Schopf, 1968; Schopf and Blacic, 1971). Bitter Springs chert-hosted microfossils are dominated by likely cyanobacterial taxa such as *Gloeodiniopsis*, *Oscillatoriopsis* and *Siphonophycus* (see Supplementary Table 1; Schopf, 1968; Schopf and Blacic, 1971), and also display three-dimensional preservation, including examples of fossil cells possibly exhibiting various stages of division (e.g., in *Glenobotrydion aenigmatis*) (see Fig. 6 in Schopf, 1968). By contrast, the mudstone biota reported here is characterized by microfossils with two-dimensional preservation, with poorly preserved features and deformed cell shapes.

These comparisons between fossils from mudstones and cherts in the Bitter Springs Group may evidence taphonomic differences that shape our interpretation of Tonian microbial evolution more broadly. The observed difference in preservation quality (and by implication diversity, since diversity metrics often rely on the preservation of fine-scale features (e.g., Anderson et al., 2024)) is likely partially due to the fossilization processes at play in each lithology. Many Proterozoic chert samples, including the Bitter Springs Group

chert (Schopf, 1968), are peritidal deposits, mainly preserving *in situ* communities such as microbial mats (Knoll, 1985; Manning-Berg et al., 2019). By contrast, mudstones likely record relatively deeper water settings. Although some benthic microfossils (e.g., *Siphonophycus*) may be preserved *in situ* in these deeper environments, a significant number of taxa likely derive from the water column. In this latter case, the dead microbes may have spent more time in oxic surface waters before being buried on the seafloor, potentially exposing them to oxic degradation *en route* (Butterfield, 1995). Enhanced degradation through greater oxidant exposure, along with compaction during burial, may lower the probability of preserving intricate and biologically diagnostic features in mudstones relative to very shallow water cherts.

Another factor that may influence observed differences in diversity and preservation quality between lithologies concerns the contrasting methods of fossil recovery and examination in cherts and mudstones. Microfossils found in chert are normally identified from thin sections. As specimens are preserved three-dimensionally, a sectional view may obscure taxonomically relevant features of a microfossil, especially if their size exceeds the thickness of the thin-section. However, for smaller microfossils where a complete specimen is encompassed by the thin-section thickness, an ability to shift the plane of focus to different levels within the section during microscopy may enhance our understanding of specimen morphology by allowing us to fully examine three-dimensional features. By contrast, for mudstone samples, acid maceration techniques are the preferred method of extraction for the purely two-dimensional microfossil specimens they preserve (e.g., Butterfield and Harvey, 2012). Although this allows for a greater volume of rock to be prospected for microfossils (e.g., Butterfield et al., 1994), and special low-manipulation procedures can be employed (e.g., Butterfield and Harvey, 2012), it is potentially more damaging to the specimens and can result in the loss of delicate surface features. These delicate features (e.g., processes), are

366 frequently used as diagnostic characters to assign microfossil specimens to specific taxa (e.g.,
367 Liu et al., 2014; Xiao et al., 2014), meaning accurate biodiversity estimates may be difficult
368 to obtain from mudstone hosted biotas.

369 The influence that host lithology exerts on microfossil preservation has previously
370 been observed in other Neoproterozoic assemblages. Another example of microfossils within
371 a single succession from both cherts and mudstones is the Tonian Svanbergfjellet Formation,
372 Svalbard (Butterfield et al., 1994). Although the Svanbergfjellet Formation has a reported
373 diversity of >30 microfossil genera, reported diversity in the chert-hosted assemblage was
374 lower than in the mudstone biota, representing only ~20% of the total diversity. In contrast to
375 the eukaryote-rich mudstone assemblage, Svanbergfjellet cherts were dominated by likely
376 cyanobacteria (e.g., *Gloeodiniopsis*, *Eoentophysalis*, *Polybessurus*, *Eosynechococcus*,
377 *Sphaerophycus*, and *Myxococcoides*). Only four genera were recovered from both the chert
378 and mudstone samples. These comprise species that are considered to have wide ecological
379 tolerances, including the acanthomorphic acritarch *Trachyhystrichosphaera aimika*, and the
380 filamentous taxa *Palaeolyngbya catenata, Rugosoopsis tenuis, Siphonophycus kestron* and
381 *Siphonophycus typicum* (Butterfield et al., 1994), and thus could be preserved in distinct
382 sedimentary settings. The enhanced diversity in Svanbergfjellet mudstones compared to
383 cherts, contrasting with the patterns documented here between Bitter Springs Group
384 mudstones and cherts, may reflect exceptional preservation in its mudstone biota (Butterfield
385 et al., 1994; Anderson et al., 2020; Mughal et al., 2025), emphasizing how local taphonomic
386 conditions can play a strong influence on the recorded diversity of Proterozoic assemblages.

387 The Ediacaran Doushantuo Formation, South China, does not preserve microfossils in
388 cherts and mudstones, but in cherts and phosphorites (Liu et al., 2014; Xiao et al., 2014).
389 Studies of early cherts have revealed >100 microfossil species of acanthomorphic and
390 spheroidal microfossils, alongside numerous cyanobacterial filaments, algal thalli, and

391 tubular structures (Liu et al., 2014; Liu and Moczydłowksa, 2019). These cherts are
392 associated with shallow shelf and lagoonal environments, with microfossils mainly found
393 from the shallow margin of the shelf lagoon (Jiang et al., 2011). By contrast, a diversity of
394 only ~50 acanthomorphic and spheroidal taxa has been reported from phosphorites (Xiao et
395 al., 2014; Wu et al., 2024), even though both lithologies preserve a similar diversity of other
396 microfossils, with a number of cyanobacterial, algal and tubular taxa common to both
397 lithologies. Furthermore, of the ~50 acanthomorph species reported in phosphorites, only 26
398 species are also preserved in the cherts (although some genera are represented by different
399 species in each lithology). It is possible that this discrepancy in the observed diversity of
400 acanthomorphs may be due to differences in ecology (Muscente et al., 2015), but the
401 extraction and recovery methods used likely also played a role, with phosphatized specimens
402 requiring acid maceration of the host rock, potentially causing damage to microfossil
403 specimens and hence a loss of diagnostic features. For example, taxa with long or delicate
404 processes (e.g., *Appendisphaera*, *Ericiasphaera*, *Mengeosphaera*, *Tanarium* and
405 *Tianzhushania*) are especially vulnerable, with many of these genera exhibiting fewer
406 recovered species in phosphorites versus cherts. In particular, Xiao et al. (2014) noted that
407 *Appendisphaera, Ericiasphaera* and *Tianzhushania* were not recoverable from phosphorite
408 macerates, only being identified from well-preserved specimens in complementary thin
409 sections.

410 Ediacaran microfossils more widely are a useful case study, as many of the
411 microfossils described from cherts and phosphorites in the Doushantuo Formation are known
412 elsewhere from mudstones in, for example, Australia (e.g., Willman and Moczydłowska,
413 2008; Grey and Willman, 2009), Estonia (e.g., Arvestål and Willman, 2020), and Siberia
414 (e.g., Moczydłowska et al., 1993; Moczydłowska, 2005). Although well-preserved examples
415 of acanthomorphic taxa have been reported from mudstones (e.g., Moczydłowska, 2005),

these fossils tend to be qualitatively more poorly preserved, with fine features being harder to observe due to taphonomic degradation resulting in loss of ornamentation, and distortion of original shape coupled with folding of the vesicle wall due to compression and loss of three-dimensionality (Grey and Willman, 2009). For example, despite the reasonably high diversity of organic-walled microfossils (38 species) reported from Estonian material, only two acanthomorphic specimens were recovered by Arvestål and Willman (2020), both of which were taxonomically unidentifiable. Additionally, although Willman and Moczydłowska (2008) report several examples of well-preserved acanthomorphic microfossils from Australian mudstones, they note that many of the microfossils they identify as leiospheres may in fact be poorly preserved acanthomorphs. Consequently, reported diversity among mudstone hosted Ediacaran assemblages lags behind that reported for the Doushantuo Formation, with a study by Zhou et al. (2007) noting how little taxonomic overlap was present in reported acanthomorphic species between Australian assemblages (mudstones) and those from South China (cherts and phosphorites). While this could be a function of contrasting extraction techniques, stratigraphy, or paleogeography, there is likely also taphonomic convergence between taxa within mudstones masking true diversity, as suggested by Zhou et al. (2007).

Taphonomic convergence of morphologies due to poorer preservation of microfossils (i.e., loss of diagnostic features) in many mudstones, when compared to cherts or phosphorites, likely contributes to an underestimation of Proterozoic biodiversity in these deposits, due to the possibility of poorly preserved specimens of different species being incorrectly grouped into a single species (e.g., *Leiosphaeridia* likely represents a polyphyletic grouping; for discussion, see Miao et al. (2019)). This may be especially true for early eukaryotes, where recognition of delicate vesicle surface ornamentation or intricate cellular features is often essential for correct taxonomic identification of otherwise morphologically

indistinguishable microfossils, and where a recent Proterozoic compilation of within assemblage eukaryote diversity identified mudstones as their dominant lithological repository: of the assemblages studied by Porter et al. (2025), 85% of those hosted in mudstones preserved eukaryotic microfossils.

However, as already noted, some mudstones do preserve microfossils with exceptional fidelity, enabling delicate diagnostic features like processes to survive (e.g., Tonian Svanbergfjellet and Wynniatt Formations) (Butterfield et al., 1994; Butterfield, 1995; Butterfield and Rainbird, 1998; Mughal et al., 2025). In these deposits, low TOC (Woltz et al., 2021) and high clay content (Anderson et al., 2020; Woltz et al., 2023; Millikin et al., 2025) may contribute to enhanced preservation quality. However, this association of high clay content likely limits the range of taxa in these exceptional mudstone hosted biotas to those with ecologies suited to environments where clay deposition was favored (e.g., paleotropics, mid-latitudes) (Anderson et al., 2018; Anderson et al., 2020).

An additional factor that may have contributed to the observed taxonomic differences between the Bitter Springs mudstone and chert assemblages is the depositional environments represented by the host strata. The early diagenetic cherts of the Loves Creek Formation formed within shallow peritidal carbonate settings characterized by microbial mat development and episodic evaporitic conditions (Hill et al., 2000; Klaebe et al., 2017). Mudstones throughout the Bitter Springs Formation evidence shallow water conditions, likely in physically protected and quiescent basins. For example, the Johnnys Creek Formation records prolonged deposition of red-bed silts and mudstones associated with salt flats and shallow saline ponds during a phase of relative sea-level fall (Hill et al., 2000; Klaebe et al., 2017). Consequently, the chert and mudstone assemblages likely sampled distinct ecological habitats in addition to preserving organisms in taphonomic pathways characteristic of their host lithologies.

The hypersaline character inferred for parts of the Bitter Springs depositional system may have exerted an important ecological control on microfossil diversity, particularly among eukaryotes which are of low diversity in its mudstones compared to other settings globally at this time (e.g., Svanbergfejellet Formation, Svalbard). Modern hypersaline environments typically host relatively low-diversity ecosystems dominated by a restricted suite of highly specialized halotolerant organisms (Ma et al., 2010). Increasing salinity specifically imposes substantial osmotic stress on eukaryotic cells, requiring specialized physiological adaptations (Ma et al., 2010; Oren, 2014). Although eukaryotes are capable of inhabiting elevated salinities, only a limited number of taxa tolerate near-saturation conditions, with communities today commonly dominated by the chlorophyte *Dunaliella* (Oren, 2014). Indeed, *Dunaliella* can become the principal or sole primary producer in modern hypersaline environments and salt lakes at the highest salinities, where other oxygenic phototrophs cannot survive (Oren, 2014). If comparable ecological filtering operated within the Bitter Springs basin, then the lower diversity of eukaryotic taxa may partly reflect genuine environmental restriction. Pairing contextual geological information on depositional environments is critical in accounting for local patterns of taxonomic composition and diversity.

CONCLUSIONS

This study demonstrates that lithology and depositional environment are correlated to both the taxonomic composition and preservation quality of microfossils in the ~800 Ma Bitter Springs Group. Its cherts capture a more diverse and well-preserved assemblage, likely reflecting rapid *in situ* silicification and exceptional taphonomic fidelity, whereas its mudstones preserve fewer taxa with poorer morphological detail. This lithological distinction in diversity is not mirrored universally across the Proterozoic fossil record: exceptional

preservation within the mudstones of some units can lead to higher diversity than in their cherts, e.g., the Svanbergfjellet Formation. In the Bitter Springs Group, eukaryotes are generally confined to mudstones but are less diverse than found elsewhere globally from that time, perhaps reflective of genuine ecological restriction in saline Bitter Springs environments. The contrast in assemblages between chert and mudstone lithologies in the Bitter Springs Group provides an example of how diversity estimates based on a single rock type and/or depositional environment from Proterozoic strata risk under-sampling microfossil diversity. Even within a single lithology, factors affecting microfossil preservation (e.g., TOC and clay content within mudstones; Woltz et al., 2021; Woltz et al., 2023) may challenge accurate comparisons of diversity sample-by-sample and between depositional environments. Consequently, studies reporting Proterozoic microfossils should aim to examine multiple lithologies from the same geological succession. This approach will both improve our characterization of the factors that influence observed trends in diversity and preservation quality across the Proterozoic, and help us to understand how their inherent biases impact our ability to effectively chart the evolutionary history of eukaryotes, both morphologically and ecologically.

ACKNOWLEDGEMENTS

This work was supported by the Department of Earth Sciences at the University of Oxford and the Royal Society (grants: URF\R1\221220 and RF\ERE\221057). We thank Z. Tian for assistance with fossil extractions, K. Sokół for SEM support, E. Howlett for collections support, and Shuhai Xiao and one anonymous reviewer for their helpful comments.

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Figure captions:

FIG. 1.—Overview of the study area in central Australia, geology of the Bitter Springs Group, and the distribution of recovered microfossils. **A**) Location of study area within Australia (modified from Klaebe et al., 2017). **B**) Selected study areas from previous investigations of the Bitter Springs Group and the location of the Wallara-1 drill core within the Amadeus basin (modified from Klaebe et al., 2017). **C**) Context of the Bitter Springs Group relative to the main eukaryotic events in the Proterozoic (adapted from Mughal et al., 2025). **D**) Generalized stratigraphy of the Bitter Springs Group, and its relationship to the Heavitree Quartz and Areyonga Formation (AF = Areyonga Formation) (modified from Klaebe et al., 2017). **E**) Stratigraphy of the Loves Creek and Johnnys Creek formations from the Wallara-1 drill core (modified from Klaebe et al., 2017). **F**) Carbonate carbon isotope chemostratigraphy of the Bitter Springs Group showing the negative excursion in the Loves Creek Formation (data from Swanson-Hysell et al., 2010). **G**) Distribution of microfossil taxa recovered from mudstone samples in this study. See Supplementary Table 2 for sample depth within core. Samples numbers (e.g., W49) are listed on the left-hand side of this panel. Horizontal dashed lined indicate relationship between core stratigraphy in (E), isotope data in (F), and fossil distribution data in (G).

FIG. 2.—Photomicrographs of filamentous microfossils recovered from Bitter Springs mudstones. In this and subsequent figures, Oxford University Museum of Natural History (OUMNH) collection numbers are given for figured specimens. **A, B**) *Oscillatoriopsis princeps* (OUMNH: PAL-ÁW.00151, PAL-ÁW.00150). **C–G**) *Palaeolyngbya catenata* (OUMNH: PAL-ÁW.00156, PAL-ÁW.00162, PAL-ÁW.00159, PAL-ÁW.00158, PAL-

ÁW.00160). **H**) ?*Pseudodendron anteridium* (OUMNH: PAL-ÁW.00152). **I–K**) *Siphonophycus* spp. (OUMNH: PAL-ÁW.00154, PAL-ÁW.00157, PAL-ÁW.00155).

FIG. 3.—Photomicrographs of spheroidal microfossils recovered from Bitter Springs mudstones. **A, B**) *Cerebrosphaera globosa* (OUMNH: PAL-ÁW.00169, PAL-ÁW.00168)**, C–J**) *Leiosphaeridia* spp. (OUMNH: PAL-ÁW.00138, PAL-ÁW.00141, PAL-ÁW.00163, PAL-ÁW.00140, PAL-ÁW.00137, PAL-ÁW.00153, PAL-ÁW.00145, PAL-ÁW.00161). A large intracellular inclusion is visible in (C), and an arrow indicates the location of several small intracellular inclusions in (H). An example of a possible medial split is exhibited in (E). Expanded view in (G) shows detail of perforations.

FIG. 4.—Photomicrographs of multicellular/colonial microfossils recovered from Bitter Springs mudstones. **A–C**) *Glenobotrydion aenigmatis* (OUMNH: PAL-ÁW.00142, PAL-ÁW.00144, PAL-ÁW.00143). An expanded view in (A) shows a cell containing two intracellular inclusions. **D–I**) *Synsphaeridium* sp. (OUMNH: PAL-ÁW.00148, PAL-ÁW.00167, PAL-ÁW.00164, PAL-ÁW.00146, PAL-ÁW.00147, PAL-ÁW.00149). Possible medial splits are exhibited in (E) and (F). An arrow indicates the location of a small intracellular inclusion in (G). **J, K**) ?*Symplassosphaeridium* sp (OUMNH: PAL-ÁW.00166, PAL-ÁW.00165).

FIG. 5.—Relationship between sample species richness and redox state (as indicated by iron speciation data from Guilbaud et al., 2015) for thirteen Bitter Springs Group mudstone samples. See Supplementary Table 2 for sample depth within core and geochemical data from Guilbaud et al. (2015).