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Brief Report

Conserved bacterial genomes from two geographically isolated peritidal stromatolite formations shed light on potential functional guilds

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Summary

Stromatolites are complex microbial mats that form lithified layers. Fossilized stromatolites are the oldest evidence of cellular life on Earth, dating back over 3.4 billion years. Modern stromatolites are relatively rare but may provide clues about the function and evolution of their ancient counterparts. In this study, we focus on peritidal stromatolites occurring at Cape Recife and Schoenmakerskop on the southeastern South African coastline, the former being morphologically and structurally similar to fossilized phosphatic stromatolites formations. Using assembled shotgun metagenomic analysis, we obtained 183 genomic bins, of which the most dominant taxa were from the Cyanobacteria phylum. We identified functional gene sets in genomic bins conserved across two geographically isolated stromatolite formations, which included relatively high copy numbers of genes involved in the reduction of nitrates and phosphatic compounds. Additionally, we found little evidence of Archaeal species in these stromatolites, suggesting that they may not play an important role in peritidal stromatolite formations, as proposed for hypersaline formations.

Introduction

Stromatolites are organo-sedimentary structures that date back between 3.4 and 3.8 billion years, forming the oldest fossils of living organisms on Earth (Dupraz *et al*., 2009; Nutman *et al*., 2016, 2019). The study of extant stromatolite analogues may help to elucidate the biological mechanisms that led to the formation and evolution of their ancient ancestors. The biogenicity of stromatolites has been studied extensively in the hypersaline and marine formations of Shark Bay, Australia, and Exuma Cay, The Bahamas, respectively (Khodadad and Foster, 2012; Mobberley *et al*., 2015; Centeno *et al*., 2016; Gleeson *et al*., 2016; Ruvindy *et al*., 2016; Warden *et al*., 2016; White *et al*., 2016; Babilonia *et al*., 2018; Wong *et al*., 2018; Chen *et al*., 2020). The presence of Archaea has been noted in several microbial mats and stromatolite systems (Casaburi *et al*., 2016; Balci *et al*., 2018; Medina-Chávez *et al*., 2019; Chen *et al*., 2020), particularly in the stromatolites of Shark Bay, where they are hypothesized to potentially fulfil the role of nitrifiers and hydrogenotrophic methanogens (Wong *et al*., 2017). Studies of freshwater microbialites in Mexico found that there were significantly more genes associated with phosphate uptake and metabolism than in other communities associated with fresh or marine waters (Breitbart *et al*., 2009) and that nitrogen fixation by heterocystous cyanobacteria, sulfur-reducing bacteria, and purple sulfur bacteria was important to the formation of these particular stromatolites (Falcón *et al*., 2007).

In the current study, we sought to investigate the metagenomes, at a genome-resolved level, of two geographically isolated (~2.8 km apart), peritidal, stromatolite formations at Cape Recife and Schoenmakerskop, on the eastern coast of South Africa. These formations are morphologically and structurally similar to fossilized phosphatic stromatolites dating back to the Great Oxygenation Event during the Paleoproterozoic era (Büttner *et al*., 2020). The stromatolite formations at Cape Recife and Schoenmakerskop have been extensively characterized with respect to their physical structure, nutrient, and chemical environment (Perissinotto *et al*., 2014; Rishworth *et al*., 2016, 2017b, 2019; Dodd *et al*., 2018) and they experience regular shifts in salinity due to tidal overtopping and groundwater seepage

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(Rishworth *et al*., 2019). Stromatolites are impacted by fluctuating environmental pressures caused by periodic inundation by seawater, which affects the nutrient concentrations, temperature and chemistry of the system (Rishworth *et al*., 2016). These formations are characterized by their proximity to the ocean, where stromatolites in the upper formations receive freshwater from the inflow seeps, middle formation stromatolites withstand a mix of freshwater seepage and marine over-topping, and lower formations are in closest contact with the ocean (Perissinotto *et al*., 2014). The stromatolite formations at Cape Recife and Schoenmakerskop are exposed to both fresh and marine water that has little dissolved inorganic phosphate and decreasing levels of dissolved inorganic nitrogen (Cape Recife: 82–9 μM, Schoenmakerskop: 424–14 μM) moving from freshwater to marine influenced formations (Rishworth *et al*., 2017a). Microbial communities within these levels therefore likely experience distinct environmental pressures, including fluctuations in salinity and dissolved oxygen (Rishworth *et al*., 2016). While carbon predominantly enters these systems through cyanobacterial carbon fixation, it is unclear how other members of the stromatolite-associated bacterial consortia influence mineral stratification resulting from the cycling of essential nutrients such as nitrogen, phosphorus, and sulfur.

However, not much is known about the microbial assemblage of these peritidal formations. It is hypothesized that the growth of peritidal stromatolites at these sites is promoted by decreased levels of wave action, higher water alkalinity, decreased levels of salinity, and decreased calcite and aragonite saturation (Dodd *et al*., 2018). Here, we investigate the metabolic potential of the microbes associated with these stromatolites and their potential role in stromatolite formation.

Results and discussion

Stromatolite formations were classified according to their tidal proximity as defined in previous studies, as 'Upper' (freshwater inflow), 'Middle', and 'Lower' (marine-influenced) (Perissinotto *et al*., 2014; Rishworth *et al*., 2016, 2017b). Samples for this study were collected at low tide from the upper stromatolites at Cape Recife and Schoenmakerskop in January and April 2018 for comparisons over time and geographic space. Additional samples were collected in April 2018 from middle and lower formations for extended comparison across the two sites (Fig. 1).

Preliminary assessment of the structure of bacterial communities in these samples using 16S rRNA amplicon sequencing (BioProject PRJNA574289) showed that microbial communities from the upper formations were distinct from those in the middle and lower formations (Supporting Information Fig. S1), but that all communities were dominated by Cyanobacteria with lesser, but notable abundances of Bacteroidetes, Alphaproteobacteria, and Gammaproteobacteria (Fig. 2A). Shotgun metagenomic sequencing was performed for all 10 sites (BioProject PRJNA612530), and binning of the metagenomic data resulted in a total of 183 bacterial genome bins (Supporting Information Table S1; Please refer to the Supporting Information Materials and Methods for additional details and find all scripts used here: https://github.com/samche42/Strom_Brief_report). The phylogenetic distribution and relative abundance thereof were approximately congruent with the 16S rRNA community profiles of each site, where Cyanobacteria were consistently dominant (36%–89%) while Alphaproteobacteria, Gammaproteobacteria, and Bacteroidia were less abundant but notable bacterial classes (Fig. 2B). Furthermore, scrutiny of all contigs from Cape Recife and Schoenmakerskop showed that none of the datasets comprised more than 1.5% archaeal genes. Two low-quality archaeal bins were retrieved from each of the lower formations and were classified as Woesearchaeales (order) and *Nitrosoarchaeum* (genus), respectively. The coverage of these genomes is among the lowest in each of the lower formation samples (Supporting Information Table S1) and would suggest a low numerical abundance of these Archaea, and it is likely that this kingdom is less important in the stromatolites found at Cape Recife and Schoenmakerskop. These results are largely in agreement with similar studies investigating freshwater microbialites from various regions of Mexico, and freshwater microbialites in Clinton Creek, Canada, which were dominated by Cyanobacteria and Proteobacteria but included less than 2% archaeal sequence (Breitbart *et al*., 2009; Centeno *et al*., 2012; White *et al*., 2015; Yanez-Montalvo *et al*., 2020) and is in contrast to communities present in hypersaline stromatolites, where Archaea comprise a large proportion of the microbial community (Wong *et al*., 2017, 2018). To identify bins common to both Cape Recife and Schoenmakerskop, we calculated pairwise average nucleotide identity (ANI) between all binned genomes using ANIcalculator (Varghese *et al*., 2015) and defined conserved bins as sharing more than 97% ANI in two or more of the sampled regions (Supporting Information Table S2; please refer to the Supporting Information Materials and Methods for additional details and find all scripts used here: https://github.com/samche42/Strom_Brief_report). Bins classified within the *Acaryochloris* and *Hydrococcus* genera were conserved across upper formations, and seven bins were conserved across the middle formations, which were classified within the *Rivularia* genus and Phormidesmiaceae and Spirulinaceae families (Fig. 2C). *Acaryochloris* species have been identified as dominant

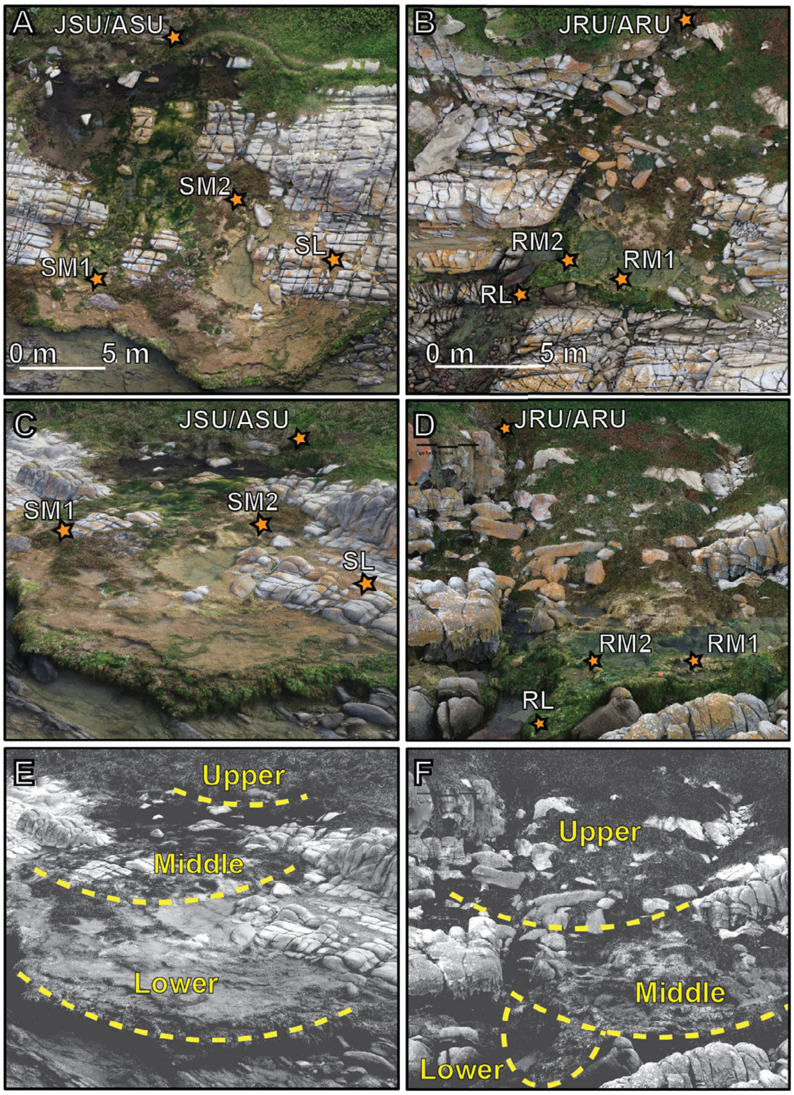


Fig. 1. Stromatolites were collected from four different points at two different sites in January and April 2017. Aerial view of sampling locations within the Schoenmakerskop site (A) and the Cape Recife site (B) is represented by orange stars. The relative elevation of the same sampling sites relative to the ocean are presented in panels C and D. A simplified schematic of delineation of 'Upper', 'Middle' and 'Lower' formations at each site are provided in panels E and F. Abbreviations are as follows: JSU: Schoenmakerskop Upper (Jan), JRU: Cape Recife Upper (Jan), ASU: Schoenmakerskop Upper April, ARU: Cape Recife Upper (April), SM1: Schoenmakerskop Middle 1 (April), SM2: Schoenmakerskop Middle 2 (April), RM1: Cape Recife Middle 1 (April), RM2: Cape Recife Middle 1 (April), SL: Schoenmakerskop Lower (April), RL: Cape Recife Lower (April).

oxygenic photoautotrophs and may promote carbonate mineralization through photosynthetically induced alkalinization in microbialite formations in the cold, fresh waters of Pavilion Lake in British Columbia, Canada (Chan *et al*., 2014; Russell *et al*., 2014; White *et al*., 2016). *Hydrococcus* species have not been reported as dominant in any stromatolite-like formations to our knowledge. However, a metagenomic study of microbialites in the Alchichica crater lake, Mexico, reported the presence of *Pleurocapsa*-like cyanobacteria surrounded by aragonite using scanning electron microscopy and provided evidence to suggest that Pleurocapsales were responsible for the biomineralization of aragonite (Saghaï *et al*., 2015). As *Hydrococcus* is within the Pleurocapsales order, it is possible that this conserved bin may be performing a similar function.

Finally, *Rivularia* spp. have been identified as key members in several stromatolite and microbialite formations, including freshwater microbialites in Pavilion Lake, Canada (White *et al*., 2016), Bacalar Lagoon, Mexico (Yanez-Montalvo *et al*., 2020), Vai Lahi Lake, Tonga (Kempe and Kazmierczak, 2012), Plateau de Langres, France (Caudwell *et al*., 2001), in microbialites formed in alkaline Lake Alchichica, Mexico (Tavera and Komárek, 1996; Kaźmierczak *et al*., 2011), and in marine

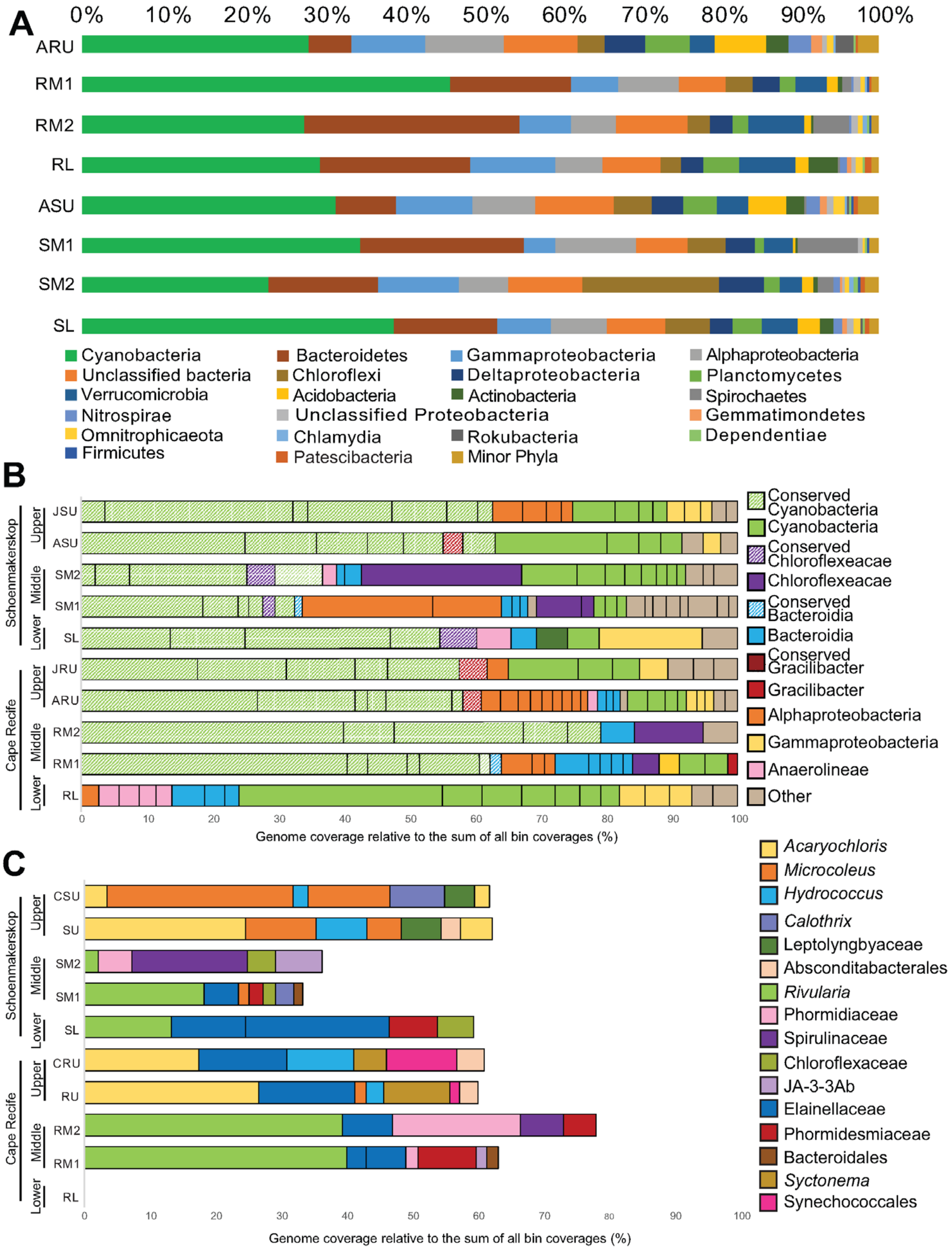


Fig. 2. Distribution and abundance of bacterial taxa in stromatolite formations (A) based on 16S rRNA gene fragment amplicon libraries. Phylogenetic classification and average relative abundance ($n = 3$) of dominant phyla in different sample sites indicated that all stromatolite samples are dominated by Cyanobacteria with notable abundances of Bacteroidetes, Alpha- and Gammaproteobacteria. OTUs are shown in relative abundance and coloured according to their phylum classification.B. Taxonomic classification of putative genome bins in stromatolites collected from Schoenmakerskop and Cape Recife. Coverage per genome has been used as a proxy for abundance (normalized to approximate genome size) and used to scale the size of individual genome bars, expressed as a percentage of the sum of all genome bin coverages. The coverage of conserved (etched lines) and non-conserved (solid colours) bacterial genomes is indicated. The taxonomic classification of each genome is indicated by colour.C. Taxonomic classifications of conserved bacterial species in each of the samples. All bins were classified using GTDB-Tk (Chaumeil *et al*., 2019). Abbreviations are as follows: JSU: Schoenmakerskop Upper (Jan), JRU: Cape Recife Upper (Jan), ASU: Schoenmakerskop Upper April, ARU: Cape Recife Upper (April), SM1: Schoenmakerskop Middle 1 (April), SM2: Schoenmakerskop Middle 2 (April), RM1: Cape Recife Middle 1 (April), RM2: Cape Recife Middle 1 (April), SL: Schoenmakerskop Lower (April), RL: Cape Recife Lower (April).

'microstromatolites' from The Bahamas, Yugoslavia, and Australia (Golubic and Campbell, 1981). Filamentous 'sister taxa' of *Rivularia*: *Calothrix* and *Dicothrix*, were similarly dominant in formations found in Highborne Cay (Myshrall *et al*., 2010; Reid *et al*., 2011). A study of the stromatolites found in hypersaline lakes in Laguna Negra, Argentina, showed the formations were dominated by *Rivularia* spp. (Shalygin *et al*., 2018), which aided in accretion through the secretion of exopolymeric substances, which trapped both inorganic matter and living organisms (such as diatoms) that contribute to stromatolite formation (Mlewski *et al*., 2018). Recent investigations into the micro-morphological features of the Cape Recife stromatolites (Büttner *et al*., 2020) revealed the presence of filamentous microbes and provided evidence to suggest that they played a key role in mineralization through the production of EPS. It is possible that the observed filamentous bacteria may be the conserved and dominant putative *Rivularia* bins identified here, and they may be contributing to the formation of the stromatolites. Furthermore, the crusts and tunnel lining surrounding these filaments have been shown to include biogenic phosphatic deposits (Büttner *et al*., 2020), and therefore it may be possible that these filamentous bacteria are responsible for the production of the phosphatic deposits.

KEGG analysis of the metagenomic datasets revealed a high abundance of genes encoding phosphate transport (*pstSCAB*), phosphate uptake regulation (*phoURBP*), alkaline phosphatases (*phoADX*), and phosphonate metabolism (*phnCDEFGHIJKLM*) (Fig. 3). Overall gene abundances indicated the negligible presence of canonical dissimilatory sulfate reduction/oxidation via *aprAB* and *dsrAB* encoded enzymes or genes associated with sulfonate metabolism (Fig. 3). Genes associated with assimilatory nitrate reduction (*narB*, *nirA*) were the most prevalent markers of nitrogen metabolism. All sites included several genes associated with dissimilatory nitrate reduction, where cytoplasmic NADH-dependent nitrate reductase *nirBD* was numerically dominant relative to periplasmic cytochrome c nitrate reductase *nrfAH* (Fig. 3).

The functional potential of each bin was assessed through KEGG annotation of genes, and gene abundance was counted and calculated as a relative percentage of total gene count per sample. Please refer to the Supporting Information Materials and Methods for additional details and find all scripts used here: https://github.com/samche42/Strom_Brief_report. As genomes investigated here were of high purity, we were confident that copy number was not an artefact of contamination. However, to ensure that this was true, we identified whether gene copies were present on the same contigs, or if found on different contigs, that the classification of the contig matched that of other copies within that bin (Supporting Information Table S3). Using this approach, we found that among conserved bins, those classified as *Acaryochloris* and *Hydrococcus* species in the upper formations included a relatively high abundance of assimilatory sulfate reduction and sulphonate metabolism, respectively (Fig. 4). Reduction of sulfate has previously been shown to promote the precipitation of carbonates in the form of micritic crusts in Bahamian and Australian stromatolites (Reid *et al*., 2000; Wong *et al*., 2018) and it has been suggested that microbial cycling of sulfur played an important role in ancient Australian stromatolites (Bontognali *et al*., 2012; Allen, 2016). Seep waters feeding the stromatolite formations at both Cape Recife and Schoenmakerskop have relatively high levels of sulfate (Dodd *et al*., 2018), and it is possible that the *Hydrococcus* and *Acaryochloris* species represented by these conserved bins may utilize this nutrient and influence the redox potential of their environment. Despite the relatively high levels of sulfate in the water, only genomes representative of a *Thioiploca* sp. from the Schoenmakerskop lower formation and a *Desulfobacula* sp. from the Schoenmakerskop middle formation included all genes required for dissimilatory sulfate reduction (Supporting Information Fig. S2). This suggests that calcite formation in these

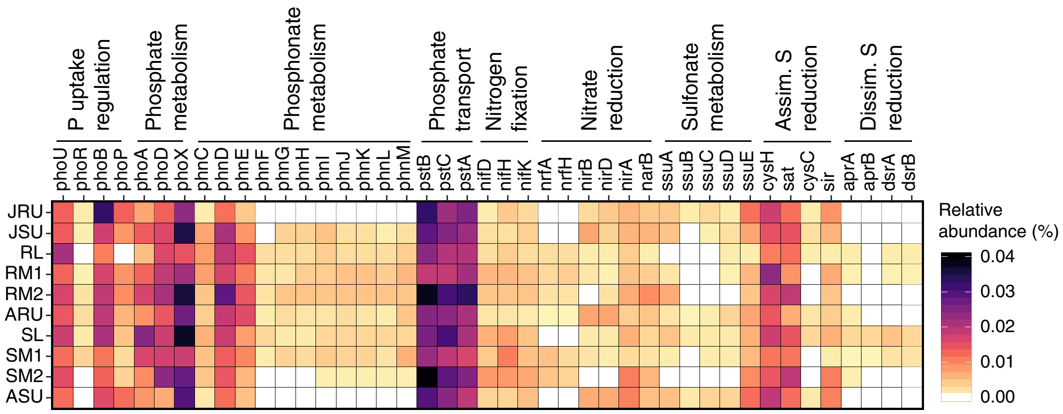


Fig. 3. The abundance of select KEGG-annotated metabolic genes relative to total gene abundance per sample site (annotated on contigs >3000 bp). Abbreviations are as follows: JSU: Schoenmakerskop Upper (Jan), JRU: Cape Recife Upper (Jan), ASU: Schoenmakerskop Upper April, ARU: Cape Recife Upper (April), SM1: Schoenmakerskop Middle 1 (April), SM2: Schoenmakerskop Middle 2 (April), RM1: Cape Recife Middle 1 (April), RM2: Cape Recife Middle 1 (April), SL: Schoenmakerskop Lower (April), RL: Cape Recife Lower (April).

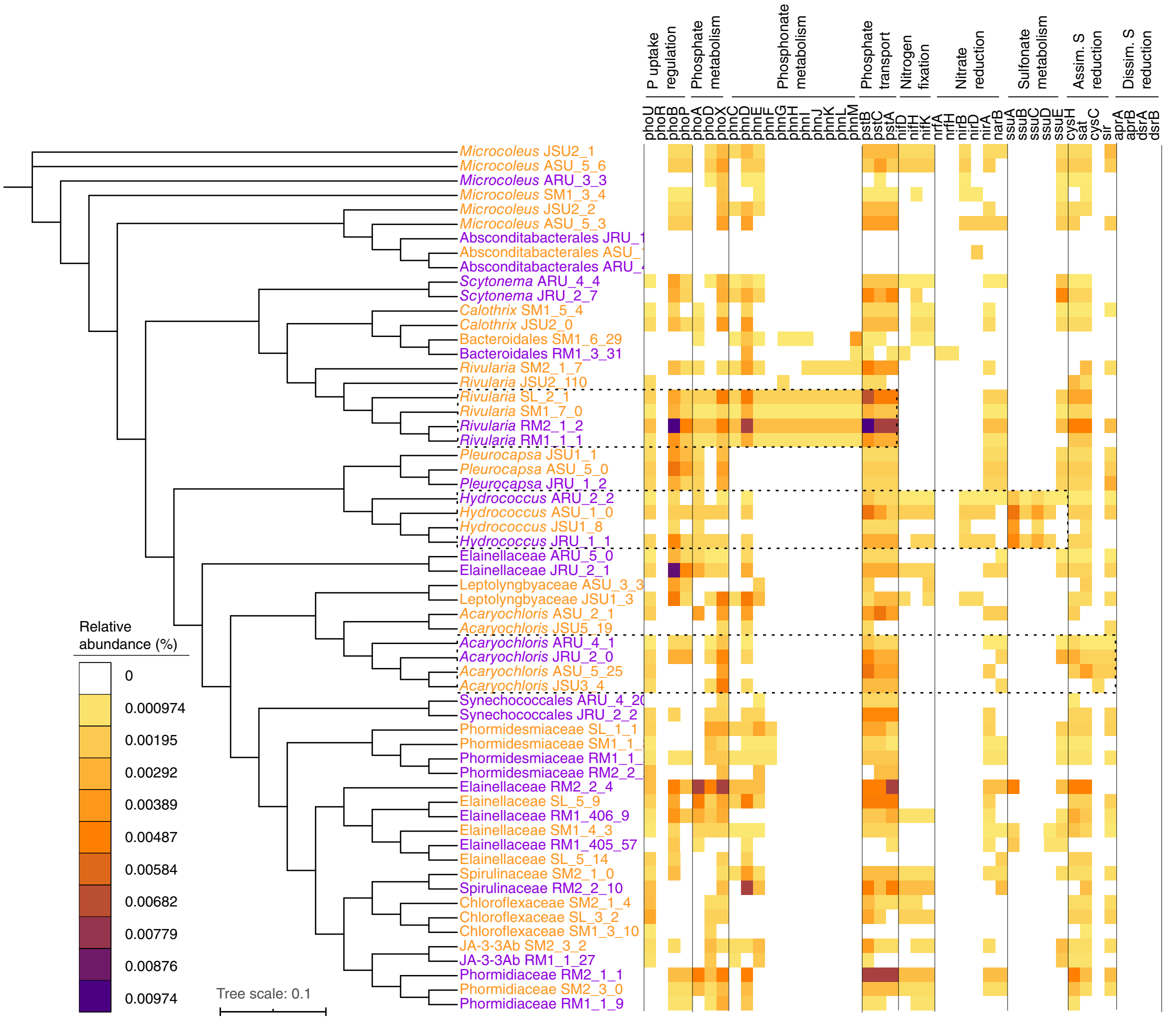


Fig. 4. Summary of phosphate, nitrogen and sulfate transport and metabolism genes in conserved bins in Schoenmakerskop and Cape Recife upper, middle, and lower stromatolite formations. Relative gene abundance was calculated as a percentage of total genes per sample. Please refer to the Supporting Information Materials and Methods for additional details and find all scripts used here: https://github.com/samche42/Strom_Brief_report. The phylogenetic tree of conserved bins was created using JolyTree (Criscuolo, 2019) with a sketch size of 10 000, and the heatmap was created in iTol (Letunic and Bork, 2019). Bins from Schoenmakerskop stromatolites are indicated with orange font, and bins from Cape Recife stromatolites are indicated with purple font. Abbreviations are as follows: JSU: Schoenmakerskop Upper (Jan), JRU: Cape Recife Upper (Jan), ASU: Schoenmakerskop Upper (April), ARU: Cape Recife Upper (April), SM1: Schoenmakerskop Middle 1 (April), SM2: Schoenmakerskop Middle 2 (April), RM1: Cape Recife Middle 1 (April), RM2: Cape Recife Middle 1 (April), SL: Schoenmakerskop Lower (April), RL: Cape Recife Lower (April). Bins were taxonomically assigned to their deepest classification as per the GTDB-Tk taxonomic classification. Numbers following underscores relate to the number of rounds of clustering performed before that bin was finalized.

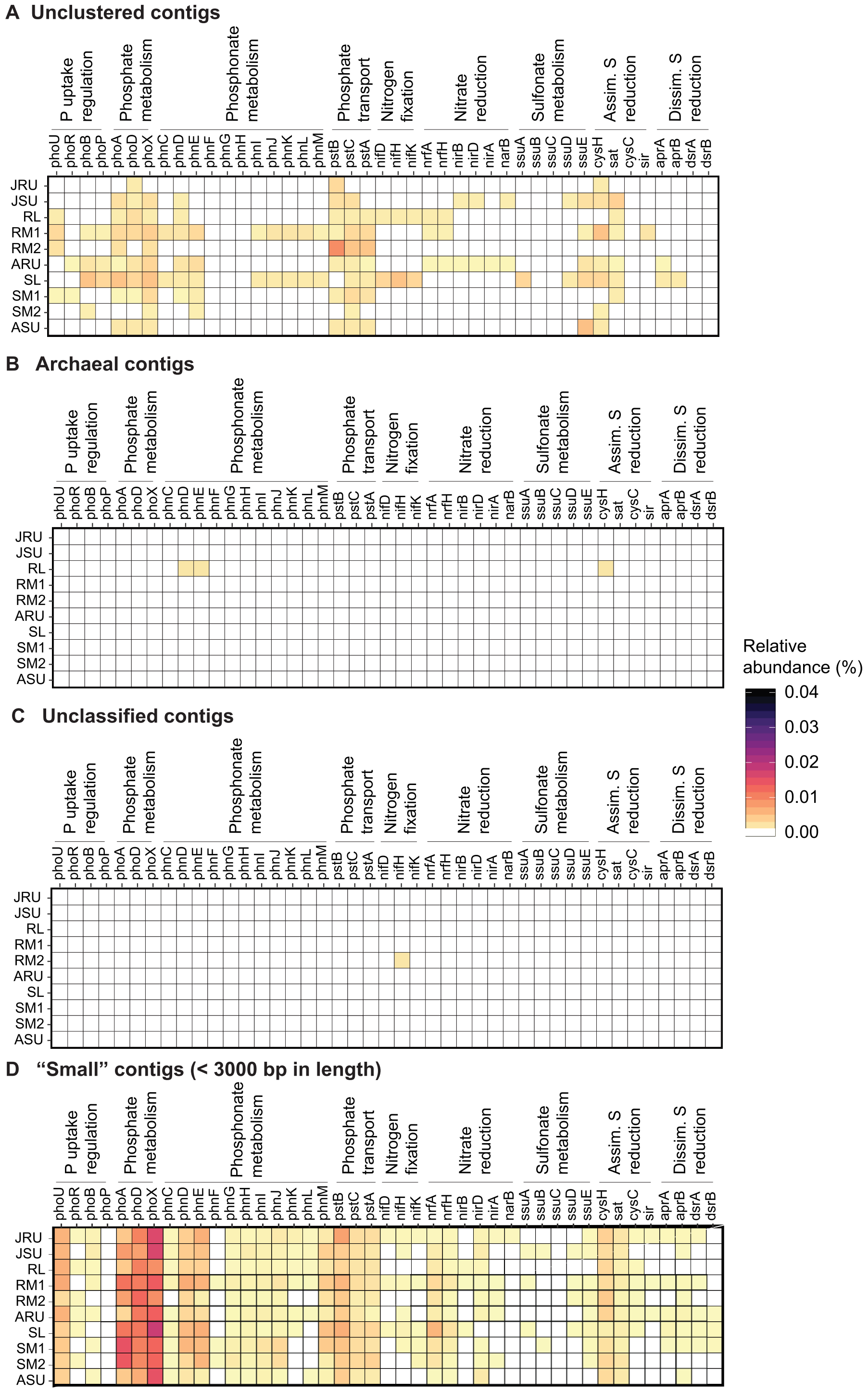
peritidal stromatolites may be influenced by processes other than dissimilatory sulfate reduction, in contrast to stromatolite formations in the Cayo Coco lagoonal network, Highborne Cay, and Eleuthera Island in The Bahamas (Visscher *et al*., 2000; Dupraz *et al*., 2004; Pace *et al*., 2018).

The reduction of nitrogen, nitrates, and nitrites can lead to calcite precipitation (Rodriguez-Navarro *et al*., 2003; Wei *et al*., 2015; Konopacka-Łyskawa *et al*., 2017; Wong *et al*., 2018; Lee and Park, 2019), and the released NH_3 can react with CO_2 and H_2O, to form $2NH^{4+}$ + CO_3^{2-} (Konopacka-Łyskawa *et al*., 2017). Therefore, bacteria that can fix nitrogen or produce ammonia from nitrates/nitrites could potentially promote the growth of stromatolites (Visscher and Stolz, 2005). We found that several of the genomic bins carried the genes associated with ferredoxin-dependent assimilatory nitrate reduction (*nirA-narB* genes) (Moreno-Vivián *et al*., 1999), nitrogen fixation (*nifDHK* genes), and dissimilatory nitrite reduction (*nirBD* genes) (Griffith, 2016), all of which result in the production of ammonia (Supporting Information Fig. S2). We noted a particularly high abundance of *nirA-narB* genes in conserved bins classified as *Rivularia* sp. (Fig. 4), which are the dominant species in the middle formations. Finally, genes associated with nitrogen fixation (*nifDHK*) were identified in several bins and were most abundant in non-conserved *Blastochloris* species (Supporting Information Fig. S2). Previous studies of nitrogen-cycling in microbialites in karstic and soda lakes in Mexico and Cuba found a large abundance of genes involved in denitrification, which originated from Alphaproteobacteria, as well as genes involved in nitrogen fixation where the majority of *nifH* transcripts (nitrogen fixation) were from bacteria within the Nostocales order (Alcántara-Hernández *et al*., 2017). However, the karstic and soda lakes were low in dissolved inorganic nitrogen (DIN) (Alcántara-Hernández *et al*., 2017), contrary to environments found in Cape Recife and Schoenmakerskop, which are relatively high in DIN, ranging from 95 to 450 mM at the two sites (Rishworth *et al*., 2016). As *Rivularia* are traditionally observed to be heterocystous nitrogen-fixers, we were surprised that we did not find any *nif* genes in the conserved *Rivularia* genomes. We additionally looked for alternative nitrogen fixation operons (*nifB*, *fdxN*, *nifS*, and *nifU* genes) (Mulligan and Haselkorn, 1989), but only identified *nifB* and *nifS* genes in the *Rivularia* bins. It is possible that these bins are incorrectly classified and may be a different species of the Nostocales order, and additional microscopy will be required for definitive classification. We further searched the non-binned bacterial contigs, contigs classified at the kingdom level as 'Archaea' and 'Unclassified', and 'small' (<3000 bp) contigs for *nif* genes (Fig. 5). We found a total of 20 *nif* genes in these 'non-binned' datasets. Upon alignment against the NCBI nr database, found that none of these 20 genes were homologous with genes from any Nostocales spp. Studies conducted around the freshwater microbialites of Cuatro Ciénegas, where DIN levels are relatively high (Breitbart *et al*., 2009), found genes associated with nitrate reduction, ammonia assimilation, and nitrogen fixation, as well as isotopic evidence for the assimilatory reduction of nitrates and nitrites but could not find isotopic signatures of nitrogen fixation (Breitbart *et al*., 2009). As the freshwater environment of Cuatro Ciénegas microbialites is somewhat similar to that found in the upper and middle formations at Cape Recife and Schoenmakerskop, it is possible that a similar trend may be found here, where nitrogen fixation is less important and the assimilatory reduction of nitrates and nitrites by conserved bins may play an important role in stromatolites.

Similarly, the bins that were classified as *Rivularia* spp. included the greatest abundance of genes associated with phosphate uptake, alkaline phosphatases, and phosphonate metabolism (Fig. 4; Supporting Information Fig. S2). The stromatolites at Cape Recife and Schoenmakerskop experience limited inorganic phosphate availability (Rishworth *et al*., 2016, 2017a, 2018; Dodd *et al*., 2018) and the high abundance of genes encoding phosphate-metabolizing enzymes may be indicative of how stromatolite communities cope with low dissolved inorganic phosphorus in their environment as suggested for microbialite assemblages in Lake Alchichica, Mexico where a similar over-representation of phosphate-associated genes was observed (Breitbart *et al*., 2009; Valdespino-Castillo *et al*., 2014). These overrepresented genes were hypothesized to play an important role in controlling carbonate precipitation through the production of stored polyphosphate, which chelates metals, such as calcium (Breitbart *et al*., 2009; Valdespino-Castillo *et al*., 2014). However, phylotyping of alkaline phosphatase genes in microbialites from Lake Alchichica suggested that Alphaproteobacteria were the possible hosts of these genes (Valdespino-Castillo *et al*., 2017), in contrast to the cyanobacterial *Rivularia* spp. as shown here. Phosphatic structures have been observed in Cape Recife stromatolites (Büttner *et al*., 2020) and due to their structural similarity to filamentous bacteria observed in tunnels lined with phosphatic deposits, and their abundant genetic potential for phosphate metabolism, the conserved *Rivularia* bins may contribute to the formation of these macrostructures.

To confirm that we had not inadvertently missed any important functional genes, we also annotated genes from contigs that were unclustered (i.e., not clustered into genome bins), small contigs (<3000 bp in length), all contigs in the 'Archaea' and 'unclassified' kingdom bins (Fig. 5). There was no specific category of predicted gene function that was hidden in these genes (e.g., dissimilatory sulfate reduction genes were confirmed as absent, rather than overlooked), but we did note an abundance of alkaline phosphatases in the 'small contigs'. A total of 373, 477, and 627 additional copies of *phoA*, *phoD*, and *phoX*, respectively, were identified, which indicated that there was even more genetic potential for inorganic phosphate production in these systems than previously realized, and further validates the hypothesis that phosphate metabolism may be important to these stromatolite formations.

Finally, we searched for genes associated with photosystem I, photosystem II, the cytochrome b6/f complex, and photosynthetic electron transport (Supporting Information Fig. S3) to identify genomes that may perform photosynthesis. Please refer to the Supporting Information Materials and Methods for additional details and find all scripts used here: https://github.com/samche42/Strom_Brief_report. A previous study of 'core' photosynthetic genes among cyanobacterial genomes found that photosystem I genes: *psaA - psaF*, *psaL*, and *psaM*, and photosystem II genes: *psbA*, *psbB*, *psbC*, *psbE*, *psbF*, *psbH*, *psbI*, *psbJ*, *psbK*, *psbL*, *psbM*, *psbO*, *psbP*, *psbT*, *psbW*, and *psbX*, and cytochrome b6 genes:



A Unclustered contigs

B Archaeal contigs

C Unclassified contigs

D "Small" contigs (< 3000 bp in length)

Fig. 5. The relative abundance of phosphate, nitrogen, and sulfate transport and metabolism genes annotated on contigs from the unclustered (A), Archaea (B), and 'Unclassified' (C) bins, as well as all contigs smaller than 3000 bp in length (D). Please refer to the Supporting Information Materials and Methods for additional details and find all scripts used here: https://github.com/samche42/Strom_Brief_report. Abbreviations are as follows: JSU: Schoenmakerskop Upper (Jan), JRU: Cape Recife Upper (Jan), ASU: Schoenmakerskop Upper April, ARU: Cape Recife Upper (April), SM1: Schoenmakerskop Middle 1 (April), SM2: Schoenmakerskop Middle 2 (April), RM1: Cape Recife Middle 1 (April), RM2: Cape Recife Middle 1 (April), SL: Schoenmakerskop Lower (April), RL: Cape Recife Lower (April).

petA - *petD*, *petG*, *petM*, and *petN*, were present in all genomes investigated (Mulkidjanian *et al*., 2006). We found a wide distribution of these genes among most of the cyanobacterial bins detected here, with particular abundance noted in the conserved *Rivularia* and *Pleurocapsa* bins (Supporting Information Fig. S3).

Cyanobacterial bins with few to no photosynthetic genes (e.g., ARU_4_12, RM1_405_57, SM2_5_0 among others) were noted as being of low quality, and absence of these genes may not be reflective of the bacterium's true genetic potential). As photosynthesis is well known to drive precipitation of carbonates (Kamennaya *et al*., 2012; Shiraishi, 2012; Zhu and Dittrich, 2016), this wide distribution and abundance of genes would suggest that photosynthesis may drive precipitation of calcium carbonate in this system, as observed in several other formations including microbial mats, stromatolites, microbialites (Reid *et al*., 2000; Dupraz and Visscher, 2005; Visscher and Stolz, 2005; Baumgartner *et al*., 2006; Kazmierczak and Kempe, 2006; Houghton *et al*., 2014; Casaburi *et al*., 2016). While there was a slight increased abundance of photosynthetic genes in *Rivularia* and *Pleurocapsa* bins, the wide distribution of the genes in several bins would suggest that carbonate precipitation is a result of microbial cumulative photosynthetic effort.

Conclusions

This pilot study has provided a glimpse into these unique, extant, stromatolite formations. In the Schoenmakerskop and Cape Recife stromatolite communities, there is extensive genetic potential for phosphate metabolism in conserved bins, classified as *Rivularia* spp., that likely contributes to the scavenging of phosphate, plays a role in the chelation of calcium ions, and aids in the control of carbonate precipitation. It is also likely that this overrepresentation of phosphate-associated genes is responsible for the accumulation of phosphatic structures in the Cape Recife stromatolite formations, and the genetic evidence here would suggest that similar mineral structures could be found in the Schoenmakerskop formations. Additionally, precipitation of calcium carbonate may be driven by photosynthesis, as there is an abundance of photosynthesis-associated genes present in the metagenomes. Genes involved in the cycling of sulfur and nitrogen were also identified in conserved bins and may influence the redox potential of the stromatolite environment. Finally, the low abundance of genes originating from Archaea would suggest that these taxa are not particularly vital to this peritidal system.

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References

Alcántara-Hernández, R.J., Valdespino, P.M., Centeno, C. M., Alcocer, J., and Falcón, L.I. (2017) Genetic diversity associated to N cycle pathways in microbialites from Lake Alchichica, Mexico. *Aquat Microb Ecol* **78**: 121–133.

Allen, J.F. (2016) A proposal for formation of Archaean stromatolites before the advent of oxygenic photosynthesis. *Front Microbiol* **7**: 1784.

Babilonia, J., Conesa, A., Casaburi, G., Pereira, C., Louyakis, A.S., Reid, R.P., and Foster, J.S. (2018) Comparative metagenomics provides insight into the ecosystem functioning of the Shark Bay stromatolites, Western Australia. *Front Microbiol* **9**: 1359.

Balci, N., Gunes, Y., Sertcetin, E., and Kurt, M.A. (2018) Non-cyanobacterial community shape the stromatolites of Lake Salda, SW, Turkey.

Baumgartner, L.K., Reid, R.P., Dupraz, C., Decho, A.W., Buckley, D.H., Spear, J.R., *et al*. (2006) Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries. *Sediment Geol* **185**: 131–145.

Bontognali, T.R.R., Sessions, A.L., Allwood, A.C., Fischer, W.W., Grotzinger, J.P., Summons, R.E., and Eiler, J.M. (2012) Sulfur isotopes of organic matter preserved in 3.45-billion-year-old stromatolites reveal microbial metabolism. *Proc Natl Acad Sci U S A* **109**: 15146–15151.

Breitbart, M., Hoare, A., Nitti, A., Siefert, J., Haynes, M., Dinsdale, E., *et al*. (2009) Metagenomic and stable isotopic analyses of modern freshwater microbialites in Cuatro Ciénegas, Mexico. *Environ Microbiol* **11**: 16–34.

Büttner, S.H., Isemonger, E.W., Isaacs, M., van Niekerk, D., Sipler, R.E., and Dorrington, R.A. (2020) Living phosphatic stromatolites in a low-phosphorus environment: implications for the use of phosphorus as a proxy for phosphate levels in paleo-systems. *Geobiology* **18**: 1–13.

Casaburi, G., Duscher, A.A., Pamela Reid, R., and Foster, J. S. (2016) Characterization of the stromatolite microbiome from little Darby Island, The Bahamas using predictive and whole shotgun metagenomic analysis. *Environ Microbiol* **18**: 1452–1469.

Caudwell, C., Lang, J., and Pascal, A. (2001) Lamination of swampy-rivulets *Rivularia haematites* stromatolites in a temperate climate. *Sediment Geol* **143**: 125–147.

Centeno, C.M., Legendre, P., Beltrán, Y., Alcántara-Hernández, R.J., Lidström, U.E., Ashby, M.N., and Falcón, L.I. (2012) Microbialite genetic diversity and composition relate to environmental variables. *FEMS Microbiol Ecol* **82**: 724–735.

Centeno, C.M., Mejía, O., and Falcón, L.I. (2016) Habitat conditions drive phylogenetic structure of dominant bacterial phyla of microbialite communities from different locations in Mexico. *Rev Biol Trop* **64**: 1057–1065.

Chan, O.W., Bugler-Lacap, D.C., Biddle, J.F., Lim, D.S., McKay, C.P., and Pointing, S.B. (2014) Phylogenetic diversity of a microbialite reef in a cold alkaline freshwater lake. *Can J Microbiol* **60**: 391–398.

Chaumeil, P.-A., Mussig, A.J., Hugenholtz, P., and Parks, D. H. (2019) GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* **36**: 1925–1927.

Chen, R., Wong, H.L., Kindler, G.S., MacLeod, F.I., Benaud, N., Ferrari, B.C., and Burns, B.P. (2020) Discovery of an abundance of biosynthetic gene clusters in Shark Bay microbial mats. *Front Microbiol* **11**: 1950.

Criscuolo, A. (2019) A fast alignment-free bioinformatics procedure to infer accurate distance-based phylogenetic trees from genome assemblies. *Riogrande Odontol* **5**: e36178.

Dodd, C., Anderson, C.R., Perissinotto, R., du Plooy, S.J., and Rishworth, G.M. (2018) Hydrochemistry of peritidal stromatolite pools and associated freshwater inlets along the Eastern Cape coast, South Africa. *Sediment Geol* **373**: 163–179.

Dupraz, C., Reid, R.P., Braissant, O., Decho, A.W., Norman, R.S., and Visscher, P.T. (2009) Processes of carbonate precipitation in modern microbial mats. *Earth Sci Rev* **96**: 141–162.

Dupraz, C., and Visscher, P.T. (2005) Microbial lithification in marine stromatolites and hypersaline mats. *Trends Microbiol* **13**: 429–438.

Dupraz, C., Visscher, P.T., Baumgartner, L.K., and Reid, R. P. (2004) Microbe-mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas). *Sedimentology* **51**: 745–765.

Falcón, L.I., Cerritos, R., Eguiarte, L.E., and Souza, V. (2007) Nitrogen fixation in microbial mat and stromatolite communities from Cuatro Cienegas, Mexico. *Microb Ecol* **54**: 363–373.

Gleeson, D.B., Wacey, D., Waite, I., O'Donnell, A.G., and Kilburn, M.R. (2016) Biodiversity of living, non-marine, thrombolites of Lake Clifton, Western Australia. *Geomicrobiol J* **33**: 850–859.

Golubic, S., and Campbell, S.E. (1981) Biogenically formed aragonite concretions in marine *Rivularia*. In *Phanerozoic Stromatolites*. Berlin, Heidelberg: Springer, pp. 209–229.

Griffith, J.C. (2016) Insights into the soil microbial communities in New Zealand indigenous tussock grasslands. PhD Thesis. University of Otago, Dunedin, New Zealand.

Houghton, J., Fike, D., Druschel, G., Orphan, V., Hoehler, T. M., and Des Marais, D.J. (2014) Spatial variability in photosynthetic and heterotrophic activity drives localized δ13C org fluctuations and carbonate precipitation in hypersaline microbial mats. *Geobiology* **12**: 557–574.

Kamennaya, N.A., Ajo-Franklin, C.M., Northen, T., and Jansson, C. (2012) Cyanobacteria as biocatalysts for carbonate mineralization. *Minerals* **2**: 338–364.

Kazmierczak, J., and Kempe, S. (2006) Genuine modern analogues of Precambrian stromatolites from caldera lakes of Niuafo'ou Island, Tonga. *Naturwissenschaften* **93**: 119–126.

Kaźmierczak, J., Kempe, S., Kremer, B., López-García, P., Moreira, D., and Tavera, R. (2011) Hydrochemistry and microbialites of the alkaline crater lake Alchichica, Mexico. *Facies* **57**: 543–570.

Kempe, S., and Kazmierczak, J. (2012) Terrestrial analogues for early planetary oceans: Niuafo'ou caldera lakes (Tonga) and their geology, water chemistry, and stromatolites. In *Life on Earth and Other Planetary Bodies*. Hanslmeier, A., Kempe, S., and Seckbach, J. (eds). Dordrecht, Netherlands: Springer, pp. 195–234.

Khodadad, C.L.M., and Foster, J.S. (2012) Metagenomic and metabolic profiling of nonlithifying and lithifying stromatolitic mats of Highborne Cay, The Bahamas. *PLoS One* **7**: e38229.

Konopacka-Łyskawa, D., Kościelska, B., Karczewski, J., and Gołąbiewska, A. (2017) The influence of ammonia and selected amines on the characteristics of calcium carbonate precipitated from calcium chloride solutions via carbonation. *Mater Chem Phys* **193**: 13–18.

Lee, Y.S., and Park, W. (2019) Enhanced calcium carbonate-biofilm complex formation by alkali-generating *Lysinibacillus boronitolerans* YS11 and alkaliphilic *Bacillus* sp. AK13. *AMB Express* **9**: 49.

Letunic, I., and Bork, P. (2019) Interactive tree of life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* **47**: 256–259.

Medina-Chávez, N.O., Viladomat-Jasso, M., Olmedo-Álvarez, G., Eguiarte, L.E., Souza, V., and De la Torre-Zavala, S. (2019) Diversity of archaea domain in Cuatro Cienegas Basin: Archaean domes. *bioRxiv*: 766709.

Mlewski, E.C., Pisapia, C., Gomez, F., Lecourt, L., Soto Rueda, E., Benzerara, K., *et al*. (2018) Characterization of pustular mats and related *Rivularia*-rich laminations in oncoids from the Laguna Negra lake (Argentina). *Front Microbiol* **9**: 996.

Mobberley, J.M., Khodadad, C.L.M., Visscher, P.T., Reid, R. P., Hagan, P., and Foster, J.S. (2015) Inner workings of thrombolites: spatial gradients of metabolic activity as revealed by metatranscriptome profiling. *Sci Rep* **5**: 12601.

Moreno-Vivián, C., Cabello, P., Martínez-Luque, M., Blasco, R., and Castillo, F. (1999) Prokaryotic nitrate reduction: molecular properties and functional distinction among bacterial nitrate reductases. *J Bacteriol* **181**: 6573–6584.

Mulkidjanian, A.Y., Koonin, E.V., Makarova, K.S., Mekhedov, S.L., Sorokin, A., Wolf, Y.I., *et al*. (2006) The cyanobacterial genome core and the origin of photosynthesis. *Proc Natl Acad Sci U S A* **103**: 13126–13131.

Mulligan, M.E., and Haselkorn, R. (1989) Nitrogen fixation (*nif*) genes of the cyanobacterium *Anabaena* species strain PCC 7120. The nifB-fdxN-nifS-nifU operon. *J Biol Chem* **264**: 19200–19207.

Myshrall, K.L., Mobberley, J.M., Green, S.J., Visscher, P.T., Havemann, S.A., Reid, R.P., and Foster, J.S. (2010) Biogeochemical cycling and microbial diversity in the thrombolitic microbialites of Highborne Cay, Bahamas. *Geobiology* **8**: 337–354.

Nutman, A.P., Bennett, V.C., Friend, C.R.L., Van Kranendonk, M.J., and Chivas, A.R. (2016) Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures. *Nature* **537**: 535–538.

Nutman, A.P., Bennett, V.C., Friend, C.R.L., Van Kranendonk, M.J., Rothacker, L., and Chivas, A.R. (2019) Cross-examining Earth's oldest stromatolites: seeing through the effects of heterogeneous deformation, metamorphism and metasomatism affecting Isua (Greenland) ~3700 Ma sedimentary rocks. *Precambrian Res* **331**: 105347.

Pace, A., Bourillot, R., Bouton, A., Vennin, E., Braissant, O., Dupraz, C., *et al*. (2018) Formation of stromatolite lamina at the interface of oxygenic-anoxygenic photosynthesis. *Geobiology* **16**: 378–398.

Perissinotto, R., Bornman, T.G., Steyn, P.-P., Miranda, N.A.F., Dorrington, R.A., Matcher, G.F., *et al*. (2014) Tufa stromatolite ecosystems on the South African south coast. *S Afr J Sci* **110**: 1–8.

Reid, R.P., Gaspar, A.P.L., Bowlin, E.M., Custals, L., and Andres, M.S. (2011) Microbialites and sediments: a 2-year record of burial and exposure of stromatolites and thrombolites at Highborne Cay Bahamas. In *STROMATOLITES: Interaction of Microbes with Sediments*. Tewari, V., and Seckbach, J. (eds). Dordrecht, Netherlands: Springer, pp. 407–425.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., *et al*. (2000) The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature* **406**: 989–992.

Rishworth, G.M., Cawthra, H.C., Dodd, C., and Perissinotto, R. (2019) Peritidal stromatolites as indicators of stepping-stone freshwater resources on the Palaeo-Agulhas plain landscape. *Quat Sci Rev* **235**: 105704.

Rishworth, G.M., van Elden, S., Perissinotto, R., Miranda, N.A.F., Steyn, P.-P., and Bornman, T.G. (2016) Environmental influences on living marine stromatolites: insights from benthic microalgal communities. *Environ Microbiol* **18**: 503–513.

Rishworth, G.M., Perissinotto, R., Bird, M.S., and Pelletier, N. (2018) Grazer responses to variable macroalgal resource conditions facilitate habitat structuring. *R Soc Open Sci* **5**: 171428.

Rishworth, G.M., Perissinotto, R., Bird, M.S., Strydom, N.A., Peer, N., Miranda, N.A.F., and Raw, J.L. (2017a) Non-reliance of metazoans on stromatolite-forming microbial mats as a food resource. *Sci Rep* **7**: 42614.

Rishworth, G.M., Perissinotto, R., Bornman, T.G., and Lemley, D.A. (2017b) Peritidal stromatolites at the convergence of groundwater seepage and marine incursion: patterns of salinity, temperature and nutrient variability. *J Mar Syst* **167**: 68–77.

Rodriguez-Navarro, C., Rodriguez-Gallego, M., Ben Chekroun, K., and Gonzalez-Muñoz, M.T. (2003) Conservation of ornamental stone by *Myxococcus xanthus*-induced carbonate biomineralization. *Appl Environ Microbiol* **69**: 2182–2193.

Russell, J.A., Brady, A.L., Cardman, Z., Slater, G.F., Lim, D.S.S., and Biddle, J.F. (2014) Prokaryote populations of extant microbialites along a depth gradient in Pavilion Lake, British Columbia, Canada. *Geobiology* **12**: 250–264.

Ruvindy, R., White, R.A., 3rd, Neilan, B.A., and Burns, B.P. (2016) Unravelling core microbial metabolisms in the hypersaline microbial mats of Shark Bay using high-throughput metagenomics. *ISME J* **10**: 183–196.

Saghaï, A., Zivanovic, Y., Zeyen, N., Moreira, D., Benzerara, K., Deschamps, P., *et al*. (2015) Metagenome-based diversity analyses suggest a significant contribution of non-cyanobacterial lineages to carbonate precipitation in modern microbialites. *Front Microbiol* **6**: 797.

Shalygin, S., Pietrasiak, N., Gomez, F., Mlewski, C., Gerard, E., and Johansen, J.R. (2018) *Rivularia halophila* sp. nov. (Nostocales, Cyanobacteria): the first species of *Rivularia* described with the modern polyphasic approach. *Eur J Phycol* **53**: 537–548.

Shiraishi, F. (2012) Chemical conditions favoring photosynthesis-induced $CaCO_3$ precipitation and implications for microbial carbonate formation in the ancient ocean. *Geochim Cosmochim Acta* **77**: 157–174.

Tavera, R., and Komárek, J. (1996) Cyanoprokaryotes in the volcanic lake of Alchichica, Puebla state, Mexico. *archiv_algolstud* **83**: 511–538.

Valdespino-Castillo, P.M., Alcántara-Hernández, R.J., Alcocer, J., Merino-Ibarra, M., Macek, M., and Falcón, L.I. (2014) Alkaline phosphatases in microbialites and bacterioplankton from Alchichica soda lake, Mexico. *FEMS Microbiol Ecol* **90**: 504–519.

Valdespino-Castillo, P.M., Alcántara-Hernández, R.J., Merino-Ibarra, M., Alcocer, J., Macek, M., Moreno-Guillén, O.A., and Falcón, L.I. (2017) Phylotype dynamics of bacterial P utilization genes in microbialites and bacterioplankton of a monomictic endorheic lake. *Microb Ecol* **73**: 296–309.

Varghese, N.J., Mukherjee, S., Ivanova, N., Konstantinidis, K.T., Mavrommatis, K., Kyrpides, N.C., and Pati, A. (2015) Microbial species delineation using whole genome sequences. *Nucleic Acids Res* **43**: 6761–6771.

Visscher, P.T., Pamela Reid, R., and Bebout, B.M. (2000) Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology* **28**: 919–922.

Visscher, P.T., and Stolz, J.F. (2005) Microbial mats as bioreactors: populations, processes, and products. In *Geobiology: Objectives, Concepts, Perspectives*. Noffke, N. (ed). Amsterdam: Elsevier, pp. 87–100.

Warden, J.G., Casaburi, G., Omelon, C.R., Bennett, P.C., Breecker, D.O., and Foster, J.S. (2016) Characterization of microbial mat microbiomes in the modern thrombolite ecosystem of Lake Clifton, Western Australia using shotgun metagenomics. *Front Microbiol* **7**: 1064.

Wei, S., Cui, H., Jiang, Z., Liu, H., He, H., and Fang, N. (2015) Biomineralization processes of calcite induced by bacteria isolated from marine sediments. *Braz J Microbiol* **46**: 455–464.

White, R.A., Power, I.M., Dipple, G.M., Southam, G., and Suttle, C.A. (2015) Metagenomic analysis reveals that modern microbialites and polar microbial mats have similar taxonomic and functional potential. *Front Microbiol* **6**: 966.

White, R.A., Chan, A.M., Gavelis, G.S., Leander, B.S., Brady, A.L., Slater, G.F., *et al*. (2016) Metagenomic analysis suggests modern freshwater microbialites harbor a distinct core microbial community. *Front Microbiol* **6**: 1531.

Wong, H.L., Visscher, P.T., White, R.A., III, Smith, D.-L., Patterson, M.M., and Burns, B.P. (2017) Dynamics of archaea at fine spatial scales in Shark Bay mat microbiomes. *Sci Rep* **7**: 46160.

Wong, H.L., White, R.A., Visscher, P.T., Charlesworth, J.C., Vázquez-Campos, X., and Burns, B.P. (2018) Disentangling the drivers of functional complexity at the metagenomic level in Shark Bay microbial mat microbiomes. *ISME J* **12**: 2619–2639.

Yanez-Montalvo, A., Gómez-Acata, S., Águila, B., Hernández-Arana, H., and Falcón, L.I. (2020) The microbiome of modern microbialites in Bacalar Lagoon, Mexico. *PLoS One* **15**: e0230071.

Zhu, T., and Dittrich, M. (2016) Carbonate precipitation through microbial activities in the natural environment, and their potential in biotechnology: a review. *Front Bioeng Biotechnol* **4**: 4.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 The relative abundance of phosphate, nitrogen, and sulfate transport and metabolism genes in all genome bins from Schoenmakerskop and Cape Recife stromatolite formations. Please refer to the Suppl. Materials and Methods for additional details and find all scripts used here: https://github.com/samche42/Strom_Brief_report. The phylogenetic tree of conserved bins was created using JolyTree (Criscuolo, 2019) with a sketch size of 10 000, and the heatmap was created in iTol (Letunic and Bork, 2019). Abbreviations are as follows: JSU: Schoenmakerskop Upper (Jan), JRU: Cape Recife Upper (Jan), ASU: Schoenmakerskop Upper April, ARU: Cape Recife Upper (April), SM1: Schoenmakerskop Middle 1 (April), SM2: Schoenmakerskop Middle 2 (April), RM1: Cape Recife Middle 1 (April), RM2: Cape Recife Middle 1 (April), SL: Schoenmakerskop Lower (April), RL: Cape Recife Lower (April).

Figure S2 The relative abundance of genes associated with bacterial photosynthesis in all genome bins from Schoenmakerskop and Cape Recife stromatolite formations. Please refer to the Suppl. Materials and Methods for additional details and find all scripts used here: https://github.com/samche42/Strom_Brief_report. The phylogenetic tree of conserved bins was created using JolyTree (Criscuolo, 2019) with a sketch size of 10 000, and the heatmap was created in iTol (Letunic and Bork, 2019). Abbreviations are as follows: JSU: Schoenmakerskop Upper (Jan), JRU: Cape Recife Upper (Jan), ASU: Schoenmakerskop Upper April, ARU: Cape Recife Upper (April), SM1: Schoenmakerskop Middle 1 (April), SM2: Schoenmakerskop Middle 2 (April), RM1: Cape Recife Middle 1 (April), RM2: Cape Recife Middle 1 (April), SL: Schoenmakerskop Lower (April), RL: Cape Recife Lower (April).

Figure S3 Supporting information

Table S1 Characteristics and taxonomic classifications of genomes bins from the upper, middle, and lower stromatolite formations from Cape Recife and Schoenmakerskop. Taxonomic classification is provided in both GTDB-Tk format and equivalent NCBI classifications.

Table S2 Conserved bacterial species are defined by shared ANI greater than 97% in stromatolite formations from Cape Recife and Schoenmakerskop. Shared ANI is provided of bin A relative to bin B, and bin B relative to bin A.

Table S3 Summary of gene copies of phosphate, nitrogen, and sulfate transport and metabolism genes annotated on contigs clustered into genome bins from Schoenmakerskop and Cape Recife stromatolite formations.

Appendix S1: Supporting information