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OOID ACCRETING DIATOM COMMUNITIES FROM THE MODERN MARINE STROMATOLITES AT HIGHBORNE CAY, BAHAMAS

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1. Introduction

Diatoms occur as common components of living microbial mats and stromatolites (Stolz, 1990; Franks and Stolz, 2009; Reid et al., 2000). In some cases, their populations may be considerable and form distinct layers (Stolz, 2001). Many species are capable of exuding extracellular polymeric substances (EPS), and benthic diatoms in particular can contribute to sediment stabilization (Winsborough, 2000; Underwood and Paterson, 2003). Diatoms have been observed in the stromatolites at Shark Bay, Australia (Awramik and Riding, 1988) and at several different stromatolitic locations in the Bahamas (Riding et al., 1991; Riding, 1994). Diatoms first appear in the rock record in the Early Jurassic (Winsborough, 2000), thus their occurrence in modern flat laminated mats and stromatolites raises questions regarding the relatedness of these structures to fossil mats and stromatolites (Awramik and Riding, 1988; Riding, 1994). Thus, understanding their role in the biogenesis of microbialites has become a paramount issue in interpreting the fossil record.

The modern marine stromatolites at Highborne Cay, Bahamas have been under investigation for over a decade (Reid et al., 1995, 2000; Paterson et al., 2008), most recently under the auspices of the Research Initiative on Bahamian Stromatolites (RIBS). An intensive and extensive monitoring program over a 3-year period (2005–2007) has provided insight into the general ecology and sedimentary processes involved in the biogenesis of these stromatolites. What has

emerged is a picture of a very dynamic system that exhibits heterogeneity on both spatial and temporal scales (Perkins et al., 2007; Eckman et al., 2008, Paterson et al., 2008). The now classic model for stromatolite biogenesis in Highborne Cay involves three different sediment laminations and their associated microbial communities: (1) accreted ooids – Type 1 mat, (2) micrite crust – Type 2 mat, and (3) fused grain layer – Type 3 mat (Reid et al., 2000). The surface layers of unconsolidated carbonate ooids are formed by the trapping and binding by EPS-secreting cyanobacteria, primarily *Schizothrix gebeleinii* (Reid et al., 2000; Stolz et al., 2001). The thin micritic crusts are produced as the result of the activity of a surface biofilm community dominated by sulfate-reducing bacteria (Visscher et al., 1998, 2000). Lastly, the fused grain layers are the result of the boring cyanobacteria (e.g., *Solentia* sp.) (MacIntyre et al., 2000; Reid et al., 2000). It is the alternation in a quasi-successional procession of the different surface mat communities and their associated sediment layers (Type 1 – Type 2 – Type 3) that results in the growth of the stromatolite (Reid et al., 2000). More recently, this rather simplistic model has been augmented by further studies of the microbial diversity (Baumgartner et al., 2006; Franks, 2007; Desnues et al., 2008; Havemann and Foster, 2008; Foster et al., 2008), and additional ooid-accreting surfaces have been recognized. Two of these ooid-accreting communities are dominated by diatoms that have been designated "yellow fur" and "pustular blanket" based on their gross morphology and color. Yellow fur is dominated by stalked diatoms, whereas pustular blanket is populated by tube-forming diatoms. We have used a combination of light microscopy, confocal scanning laser microscopy (CSLM) enhanced by immunohistochemistry, and transmission electron microscopy (TEM) in concert with field observations and conclusions from previous related studies to assess the potential contribution of diatoms to stromatolite biogenesis.

2. Stalked Diatoms

Stalked diatoms can be found throughout the year at Highborne Cay as a minor component of the different surface communities. During the summer and early fall, however, their populations may grow and develop into a conspicuous thick layer (0.5–1cm) on the surface of the stromatolites (Fig. 1a). The community is comprised primarily of the stalked diatoms *Licmophora remulus*, *L. paradoxa*, and *Striatella unipunctata* (Fig. 1b). Although the cells of the two species of *Licmophora* (baseball bat-shaped) differ from that of the *Striatella* (pillow-shaped), they all produce a similar type of stalk. We have found that these stalks bind a lectin–FITC conjugate (Concanavalin A) as demonstrated for the stalked marine diatom *Achnanthes longipes* (Wustman et al., 1997). Conjugate binding is also not sensitive to acid treatment, such that the carbonate ooids can be removed from the mat material (Fig. 1b). Figure 1b shows an example of a yellow fur that has colonized the surface of a Type 1 mat. The individual cells of the diatoms appear to float in the overlying surface waters, tethered to the surface by their stalks.
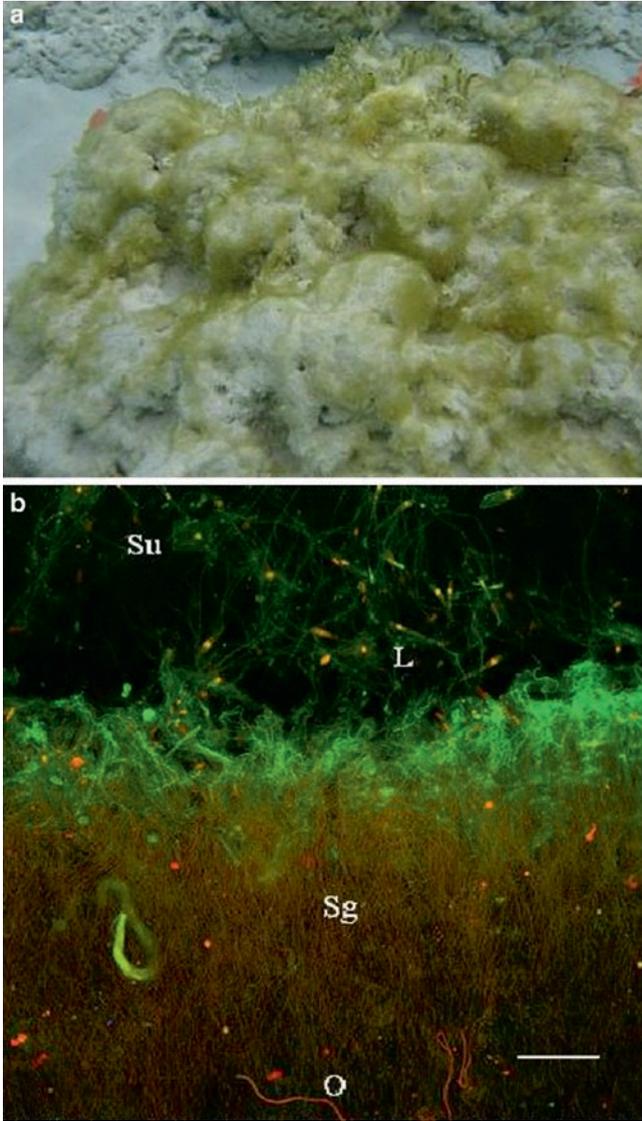
Figure 1. The "yellow fur" surface community. (**a**) A stromatolitic bioherm with an extensive surface mat of stalked diatoms which impart the yellow color. (**b**) The surface community in cross section. The sample has been acid treated (HCl) to remove the ooids, then stained with lectin–FITC conjugate to reveal the layer of stalks (green). Cells of *S. unipunctata* (Su) and *Licmophora* spp. (L) can be seen at the top of the micrograph. Filaments of *Schizothrix gebeleinii* (Sg) form a distinct layer below the stalks. A few filaments of *Oscillatoria* sp. (O) are also present. CLSM, bar 200 μm.

The surface is a dense weave of stalks almost a quarter centimeter in thickness. Immediately below this, the stalks interweave with filaments of *S. gebeleinii* for about another quarter centimeter depth (Fig. 1b). The filaments of *S. gebeleinii* appear to be oriented perpendicular to the surface possible indicating an upward migration towards the surface. The layer of *S. gebeleinii* persists for another centimeter down section, with little evidence of the diatom stalks. Individual filaments of *Oscillatoria* sp. were found further down in the mat (Fig. 1b).

The cells of *Licmophora* readily attach to surfaces and individual filaments of cyanobacteria and red algae (Fig. 2a). Initially, the diatom attaches via a holdfast region, but as the stalk grows, the cell extends away from the surface to which it is attached (Franks, 2007). Manual manipulations of the yellow fur material indicate that ooids adhere to both holdfast and stalk (Fig. 2a inset). Samples of stromatolites collected in July 2007 with well-developed yellow fur had several millimeters of trapped but unconsolidated ooids at the surface (Fig. 2b). Although there was some course-grained material (e.g., coral skeletal fragments), the bulk of the trapped sediment were fine- to medium-sized ooids (Fig. 2). This is in contrast to the reports that eualgal/cyanobacterial stromatolites are coarse-grained (Awramik and Riding, 1988; Riding, 1994). A conspicuous feature was the presence of ooid-free gelatinous pockets both near the surface and well into the subsurface (Fig. 2b, c). Lectin–FITC conjugate staining revealed that the voids were actually bundles of stalks (Fig. 2b, c). No diatom cells or their frustules were evident, indicating that they had either detached and remained at the surface, or had degraded upon burial. In some cases, the bundles of stalks became selectively colonized by filaments of *Oscillatoria* sp. (Fig. 2b inset). This raises the intriguing possibility that the stalks are providing a conduit for light into the subsurface of the stromatolite. Alternatively, the degrading stalks could be a source of nutrients. More importantly, the persistence of the stalks in the subsurface for extended periods of time (e.g., months) suggests that they could contribute to the overall integrity of the layer.
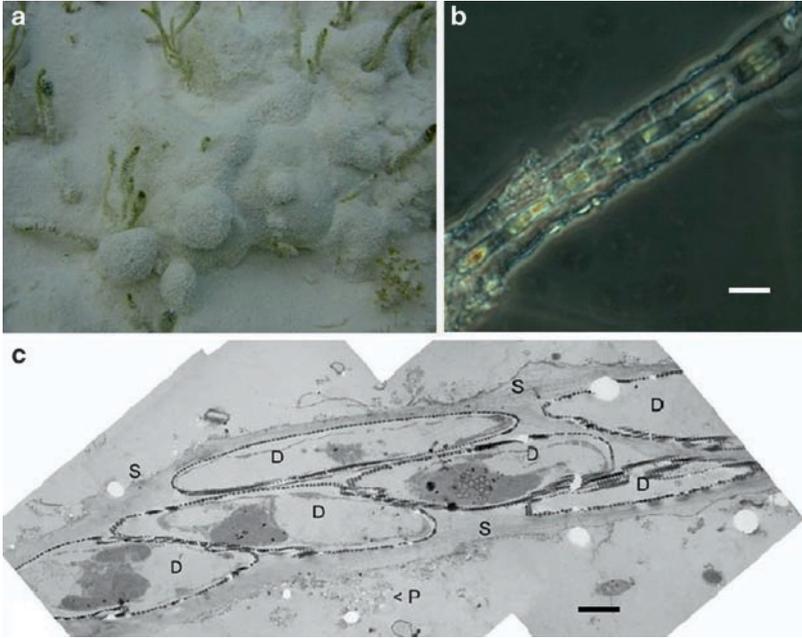
3. Tube-Forming Diatoms

Pustular blanket, the colorless cohesive surface layer, is usually seen in the fall and winter. It begins as small individual pustules (Fig. 3a), eventually coalescing into a blanket covering the surface. The identity of the organism responsible for these unique accretionary structures was not initially obvious, as dissection of the pustules often indicated only the presence of empty sheaths. However, further observation of freshly obtained material revealed the presence of a tube-forming diatom with individual cells resembling *Navicula* (Fig. 3b). The diatom is light sensitive and highly motile. Often aligned end to end inside the sheath (Fig. 3b), when disturbed (e.g., under the intense light of the microscope) the chain constricts and the cells overlay each other (Fig. 3c). The cells rapidly evacuate the sheath, leaving behind an empty tube. Ooids readily adhere to the sheath material, giving the appearance of a string of pearls. There is also evidence for carbonate
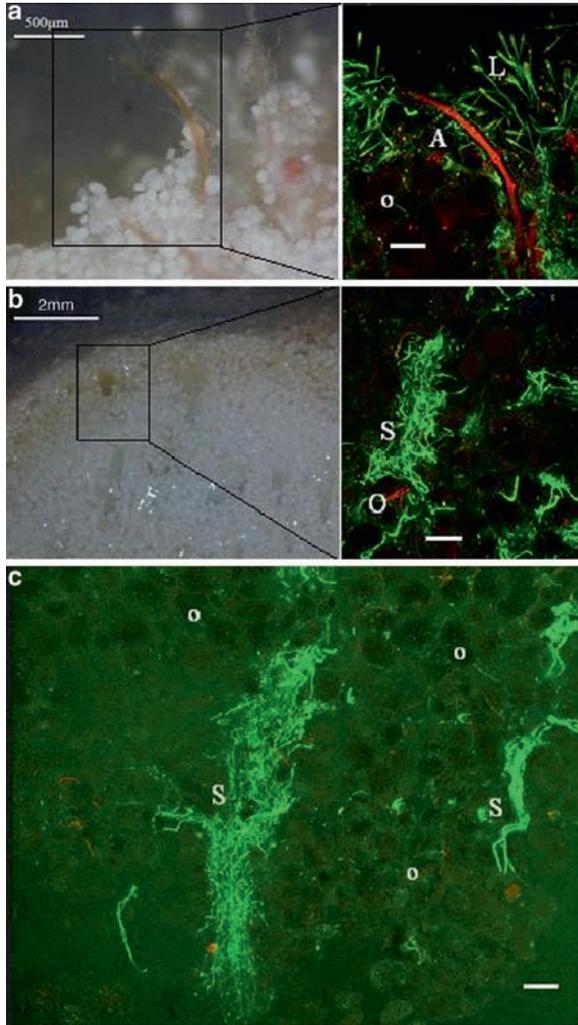
Figure 2. Stalks and ooid accretion. (**a**) *Licmophora* spp. stalks and cells (L) adhering to a red algal filament (A) and ooids (o) (inset, CLSM), (**b**) gelatinous voids in the subsurface are actually clusters of diatom stalks (S), which often get colonized by *Oscillatoria* sp. (O) (*inset*, CLSM), (**c**) clusters of diatom stalks surrounded by ooids (o) further down section (CLSM). Stalks were stained with FITC–lectin conjugate. All bars 100 μm.

precipitation associated with the sheath (Fig. 3c). The lectin–FITC conjugate did not readily bind to the sheath indicating that the composition of the EPS is different than that of the stalks of *Licmophora* and *Striatella*.

Figure 3. Pustular blanket and tube-forming diatom. (**a**) Distinctive surface accretions of "pustular blanket," (**b**) chain of *Navicula*-like diatoms inside a common sheath (tube). Phase contrast light microscopy, bar 10 μm, (**c**) cross section through chain of tubular diatoms (D) in a common sheath (S). Note precipitation (P) associated with the sheath. TEM, bar 1 μm.

4. Diatoms and Stromatolite Biogenesis

Previous reports have shown that stromatolites at Highborne Cay, Bahamas, can be produced solely by bacterial processes (Reid et al., 2000). In particular, micrite crusts are formed by bacterial biofilms (Type 2 mats) and the fused grain layers by endolithic cyanobacterial communities (Type 3 mats; Visscher et al., 1998, 2000; Macintyre et al., 2000). In addition, layers of unconsolidated sand grains are formed as a result of the trapping and binding of cyanobacteria (Type 1 mats). Nevertheless, the presence of diatoms often as conspicuous surface communities (e.g., yellow fur) raises the question of what if any role do the diatom communities described above play in stromatolite accretion?

Several field observations suggest that physical conditions at the Highborne Cay reef complex restrict the growth of diatom communities. The reef complex (including the stromatolites) is located in a high-energy environment (the surf zone) and is subject to strong winds, periods of intense wave action, and burial events (Eckman et al., 2008; Paterson et al., 2008). Both yellow fur and pustular

blanket are readily eroded by wave action. In addition, highly mobile sand bars move across the reef crest and reef flat frequently, burying the stromatolites under tens of centimeters of sediment (Andres and Reid, 2006). The duration of the burial events varies with the relative location in the reef complex (Gaspar, 2007). These burial events are key to the survival and proliferation of the stromatolites as burial inhibits the growth of macroalgae (e.g., *Batophora*), and boring macro-fauna (Andres and Reid, 2006). Burial events also impact the diatom populations, which are extremely sensitive to perturbations. In one set of experiments, the photosynthetic activity of the yellow fur community was unrecoverable after 7 days of burial (Perkins et al., 2007). In contrast, the cyanobacteria are more resil-ient and survive prolonged burial (Kromkamp et al., 2007). The photosynthetic activity in cyanobacterial-dominated mats (e.g., Type 1 mat) buried for 5 days was reactivated within 1–2 h after exposure (Kromkamp et al., 2007), and even those buried for over 2 weeks quickly rebounded (Perkins et al., 2007).

The occurrence of the surface diatom communities as "transient blooms" at specific times of the year, their susceptibility to erosion by wind and wave action, and the fact that diatoms do not survive burial, suggest that the contribution of diatoms to the growth of stromatolites in the Highborne Cay reef system may be minimal. Nevertheless, stalked diatom mats have been observed attached to a variety of stromatolite surfaces including Mat Types 1, 2, and 3. These stalked mats trap ooids, resulting in rapid and significant accretion. The tube-forming diatoms are also capable of trapping sediment and form extensive surfaces of pustular blanket. Thus both communities could form layers of accreted ooids. Although no evidence of diatom frustules are found at depth (Stolz et al., 2001), silica is undersaturated in seawater and the frustules should readily dissolve after the death of the diatom. Thus, recognition of diatom-accreted sediment in the stromatolite subsurface is difficult. Additional work involving pigment accretion studies and detailed grain size analyses is being conducted in an effort to deter-mine the amount of diatom-trapped sediment that is incorporated into the stro-matolite subsurface.

5. Acknowledgments

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