Microbiology[1]

Expedition 310 Scientists[2]

Chapter contents

Introduction

Previous research on drill cores from the barrier reef-edge of Tahiti revealed the presence of microbialites, which are generally interpreted as forming during the last stage of encrustation over dead coral colonies. Microbialites are a major structural component of the reef sequence, contributing as much as 80% of the reef framework (Camoin and Montaggioni, 1994; Camoin et al., 1999). Microbialites are, based on the definition of Burne and Moore (1987), "organosedimentary deposits that have accreted as a result of a benthic microbial community trapping and binding detrital sediment and/or forming the locus of mineral precipitation." Although Tahiti microbialites show a strong relationship between microbial activity and mineral precipitation, little is known about the microbial mechanisms associated with their formation. Neither a mineralogical nor sedimentological approach can provide precise information concerning the community of microorganisms that is involved in the processes associated with this unusual formation of macroscale structures. In order to have a better understanding of the microcosms of the Tahiti reef environments, it is important to verify whether there are still-living microorganisms preserved in reefs (i.e., survivors of those responsible for the formation of microbialites). If they are found to be present, the next questions are what types of microorganisms are present and in what abundances, and which of them are actually actively participating in the biogeochemical processes?

Visual observations

Photographs of core pieces with cavities show evidence of microbial activity (Fig. **F1**).

ATP activity

Microbial activity depth profiles, measured by adenosine 5′-triphosphate (ATP) monitoring (expressed in relative light units [RLU]) on surfaces/cavities, are shown in Figure **F2.** The high activity in Hole M0020A at 0.55 meters below seafloor (mbsf) possibly originates from eukaryotes (fungi, algae, and protozoans).

[1] Expedition 310 Scientists, 2007. Microbiology. *In* Camoin, G.F., Iryu, Y., McInroy, D.B., and the Expedition 310 Scientists. *Proc. IODP,* 310: Washington, DC (Integrated Ocean Drilling Program Management International, Inc.). doi:10.2204/iodp.proc.310.111.2007
[2] **Expedition 310 Scientists' addresses.**

doi:10.2204/iodp.proc.310.111.2007

Exoenzyme activity

Exoenzyme activity is shown in Table **T1.**

In two samples from Hole M0020A with brownish biofilms, moderate to high phosphatase activities were measured. Other exoenzymes were absent or were very low in activity.

In Hole M0005E, the extraordinary high activity in near-surface samples possibly originates from eukaryotic organisms living near the sediment surface that were mixed in the sediment by the hammer-coring method applied in Hole M0005E.

Microscopy

Light microscopic fluorescence/transmission light pictures of 4′,6-diamidino-2-phenylindole (DAPI)–stained samples are shown in Figure **F3.**

Scanning Electron Microscope

Scanning electron microscope (SEM) micrographs of glutaraldehyde-fixed samples are shown in Figure **F4.**

Principal results

Samples were analyzed for evidence of microbial activity, possibly related to the formation of microbialites. To date, onboard measurements have shown a certain degree of microbial activity, directly attached to mineral surfaces, which could be related to microbialite formation. According to activity measurements taken along the drill cores, the uppermost subsurface, 0–4 mbsf, is the most active zone. This is a common trend in reef environments because of the closeness to the photic zone inhabited by primary producing eukaryotes such as algae. Pure microbiological activity was only observed in reef cavities where prokaryotic biofilms have the right conditions to develop (Fig. **F1H, F1I**).

Preliminary results show that biofilms are diverse in structure and color. Figure **F1H** shows an association between a brown iron/manganese crust and biofilm, and Figure **F1I** shows an unusual blue biofilm, which exhibited the highest degree of ATP activity (20,600 RLU). In this sample, it was possible to define spherical assemblages of carbonate minerals embedded into the microbial exopolymeric substances (EPS). Figure **F3L** shows DAPI-stained cells in high densities in this biofilm in conjunction with mineral precipitation, which is assumed to be calcium carbonate.

Biofilms appear to have high diversity in macroscale observations, and they are equally diverse and heterogenous in microscale resolution, as observed by SEM microscopy. Carbonate minerals appear to be closely associated with claylike minerals (Hole M0007C; Fig. **F4D**), carbonate microfossils (Hole M0015B; Fig. **F4E**), and oxidized and reduced Fe minerals, such as pyrite (Hole M0023A; Fig. **F4B**). The metabolic processes responsible for the precipitation of these minerals cannot be defined as yet because of the complex and diverse conditions in the microenvironments where these biofilms were found.

Some evidence for heterotrophic metabolic activity is shown by exoenzyme measurements, which vary in different biofilm samples. For instance, samples from Holes M0020A, 4.51 mbsf, and M0009D, 3.64 mbsf, show high phosphatase activity, which suggests that a heterotrophic community preferentially degrades organic-bound phosphate compounds, such as phospholipids or nucleic acids. In contrast, samples from Hole M0007B, 6.28 mbsf, show only glucosidase and aminopeptidase activity, which is evidence for the degradation and metabolization of polysaccharides and proteins.

Isolation of microorganisms from biofilm samples was performed on agar plates using a medium that is selective for heterotrophic bacteria. After 2 weeks incubation time, 10 different heterotrophic colonies were isolated (Figs. **F5, F6;** Table **T2**). From the anaerobic experiments, only one isolation was successful. Distinct groups of microorganisms are associated with the biofilm and may represent aerobic to anaerobic metabolism. SEM investigation of microbialite samples has shown evidence that anaerobic conditions must have prevailed at times. The occurrence of framboidal pyrite, well distributed in the sediment, supports a certain degree of anoxia in the environment. Some sediment samples also show a close spatial association between the mineral phase and microbes (e.g., Figs. **F3J, F4E**).

As a biogeographical summary of microbial activity in the Tahiti reefs, northwestern Faaa Hole M0020A and southwestern Maraa Holes M0005C, M0007B, M0007C, M0015B, and M0018A were more active than the northeastern Tiarei sites, where in many cases no living biofilm were detected in cavities along the cores.

References

Burne, R.V., and Moore, L.S., 1987. Microbialites: organosedimentary deposits of benthic microbial communities. *Palaios,* 3:241-254.

Camoin, G.F., and Montaggioni, L.F., 1994. High energy coralgal-stromatolite frameworks from Holocene reefs

(Tahiti, French Polynesia). *Sedimentology,* 41(4):655–676. **doi:10.1111/j.1365-3091.1994.tb01416.x**

Camoin, G.F., Gautret, P., Montaggioni, L.F., and Cabioch, G., 1999. Nature and environmental significance of microbialites in Quaternary reefs: the Tahiti paradox. *Sediment. Geol.,* 126(1–4):271–304. **doi:10.1016/S0037-0738(99)00045-7**

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Figure F1. A. Dark-colored cavities in the reef framework, covered with microbial biofilms (Hole M0023B, 4.6 mbsf). **B.** Brown-colored cavity in the reef framework (Hole M0023B, 18.6 mbsf). **C.** Core originated from a reef cavity containing some coral branches overgrown by thrombolitic microbialites (Hole M0007C, 31.44 mbsf). **D.** Fossil coralline algal crust overgrown by microbial material (Hole M0007C, 9.75 mbsf). (Continued on next page.)

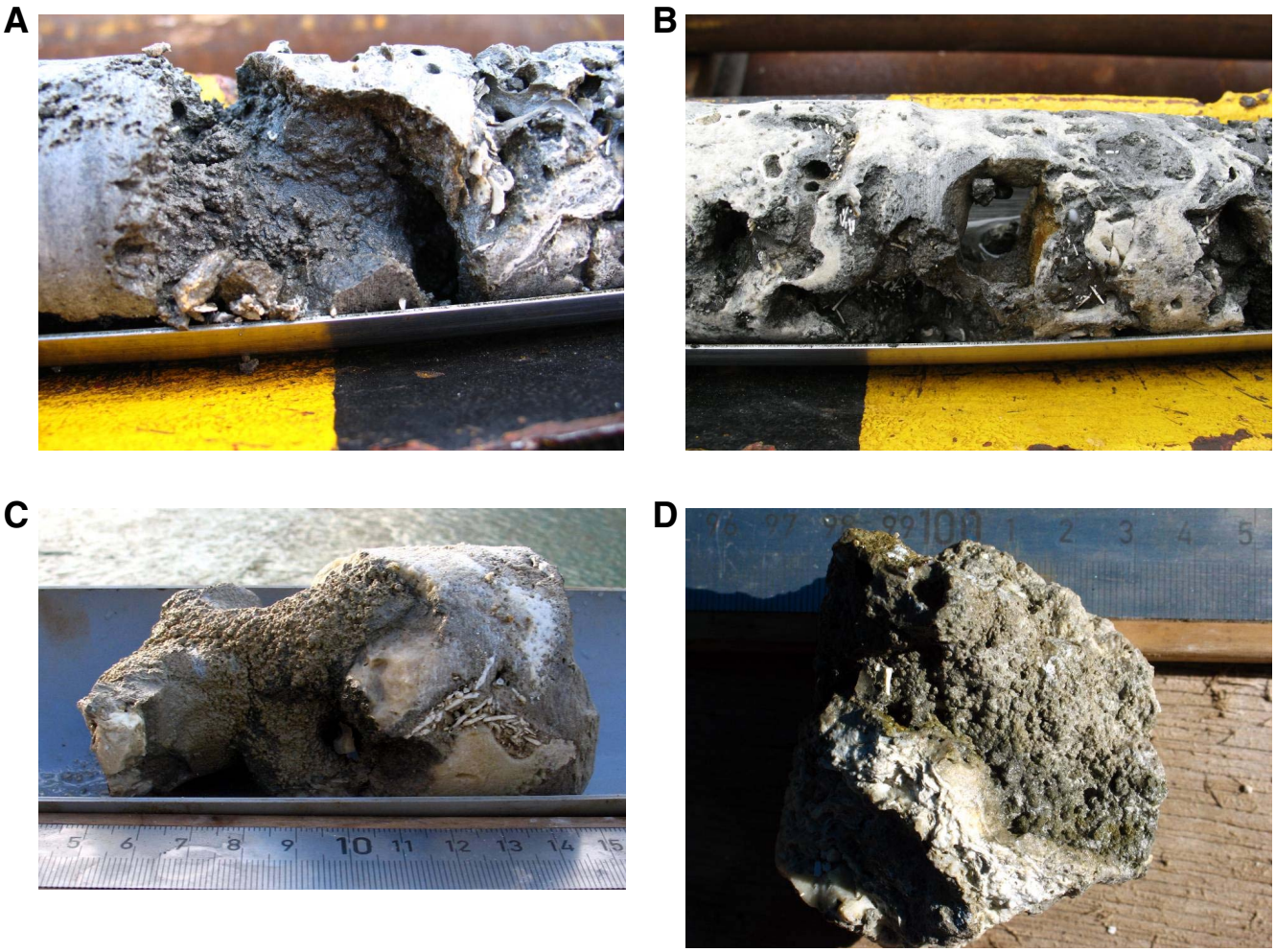

Figure F1 (continued). **E.** Core with cavity and some branching corals (Hole M0018A, 4.61 mbsf). **F.** Surface of microbialite structures with dark brown manganese crust (Hole M0015A, 29.17 mbsf). **G.** Surface of microbialite structures with a brown iron/manganese crust (Hole M0016A, 7.74 mbsf). **H.** Cavity with brown iron/manganese crust (Hole M0016B, 23.68 mbsf). **I.** Blue/purple-colored biofilm with high microbial activity and high abundance of cells (Hole M0015B, 0.47 mbsf).

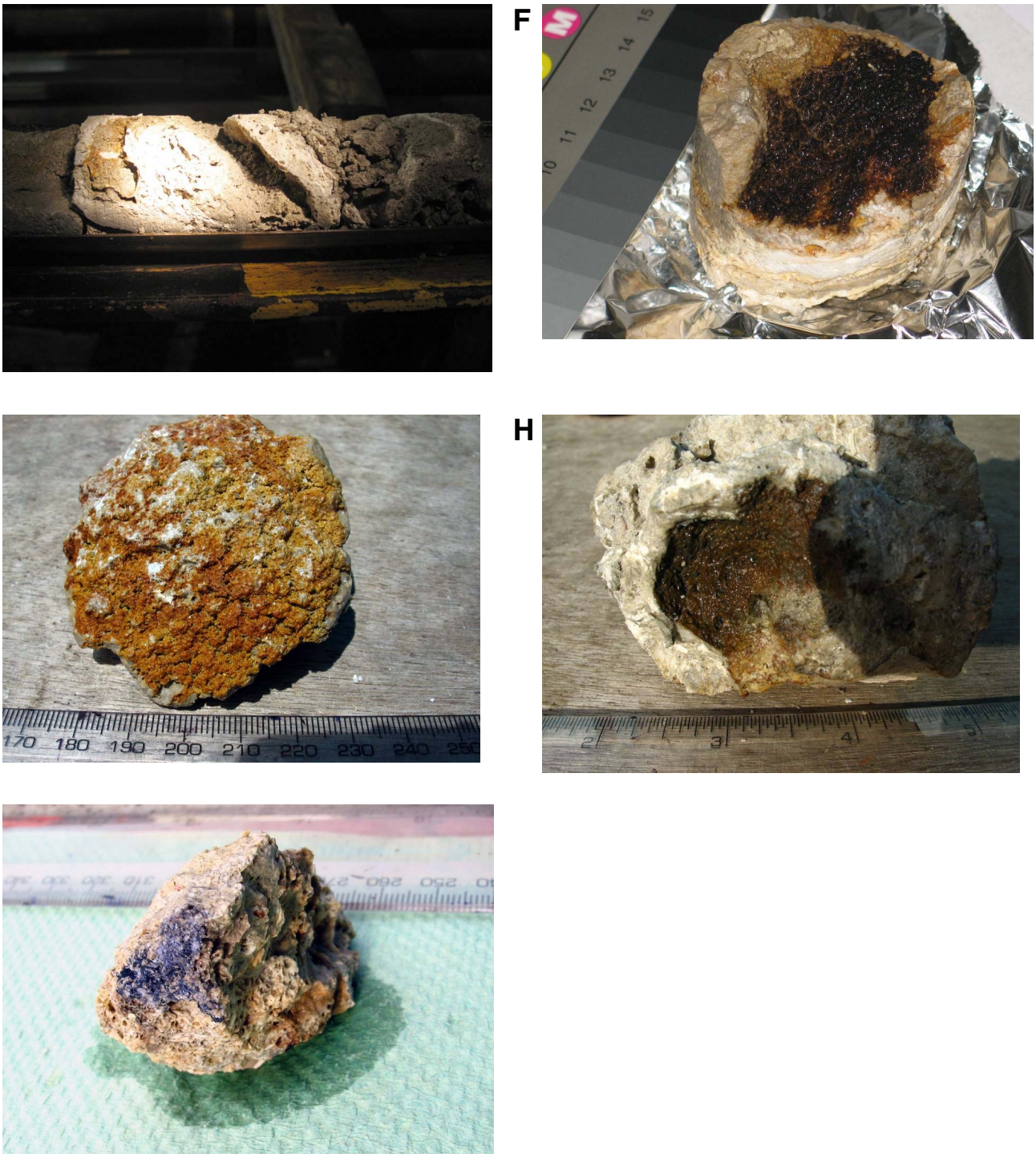

Figure F2. Microbial activity depth profile of surfaces/cavities, expressed by adenosine 5′-triphosphate (ATP) monitoring (relative light units [RLU]). At the other sites not listed here (Sites M0008, M0010, M0013, and M0014), material was not appropriate to measure ATP activity (e.g., in basaltic sands). Overall, there was almost no microbial activity detected in the recovered cores. **A.** Hole M0019A. **B.** Hole M0020A. **C.** Holes M0023A (blue) and M0023B (pink). **D.** Hole M0009D. (Continued on next two pages.)

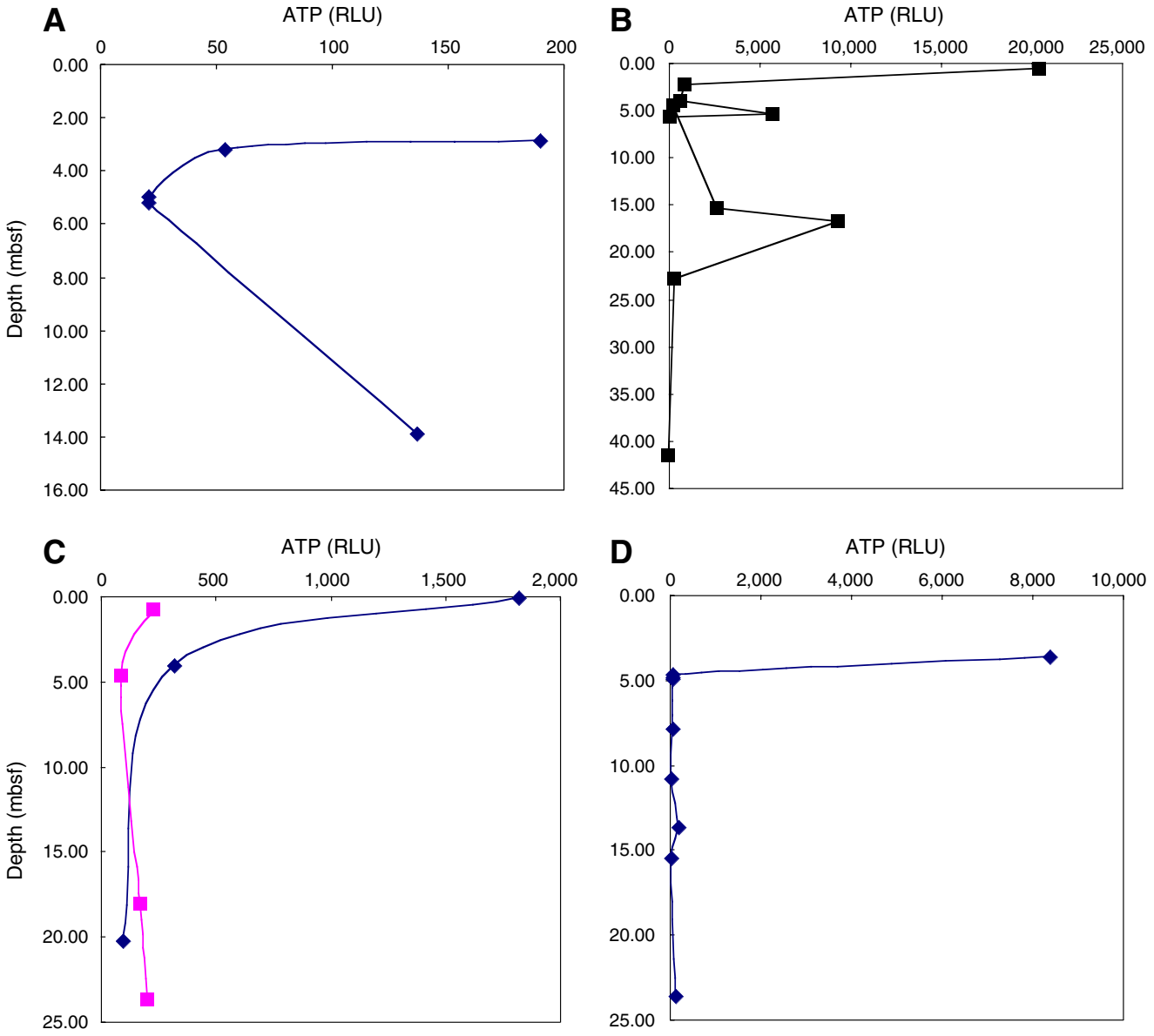

Figure F2 (continued). E. Holes M0021A (blue), M0024A (pink), and M0025A (black). **F.** Holes M0011A (blue), M0012A (pink), and M0022A (green). **G.** Holes M0005A (dark blue), M0005B (pink), M0005C (red), and M0005E (light blue). **H.** Holes M0007A (blue), M0007B (pink), and M0007C (green).

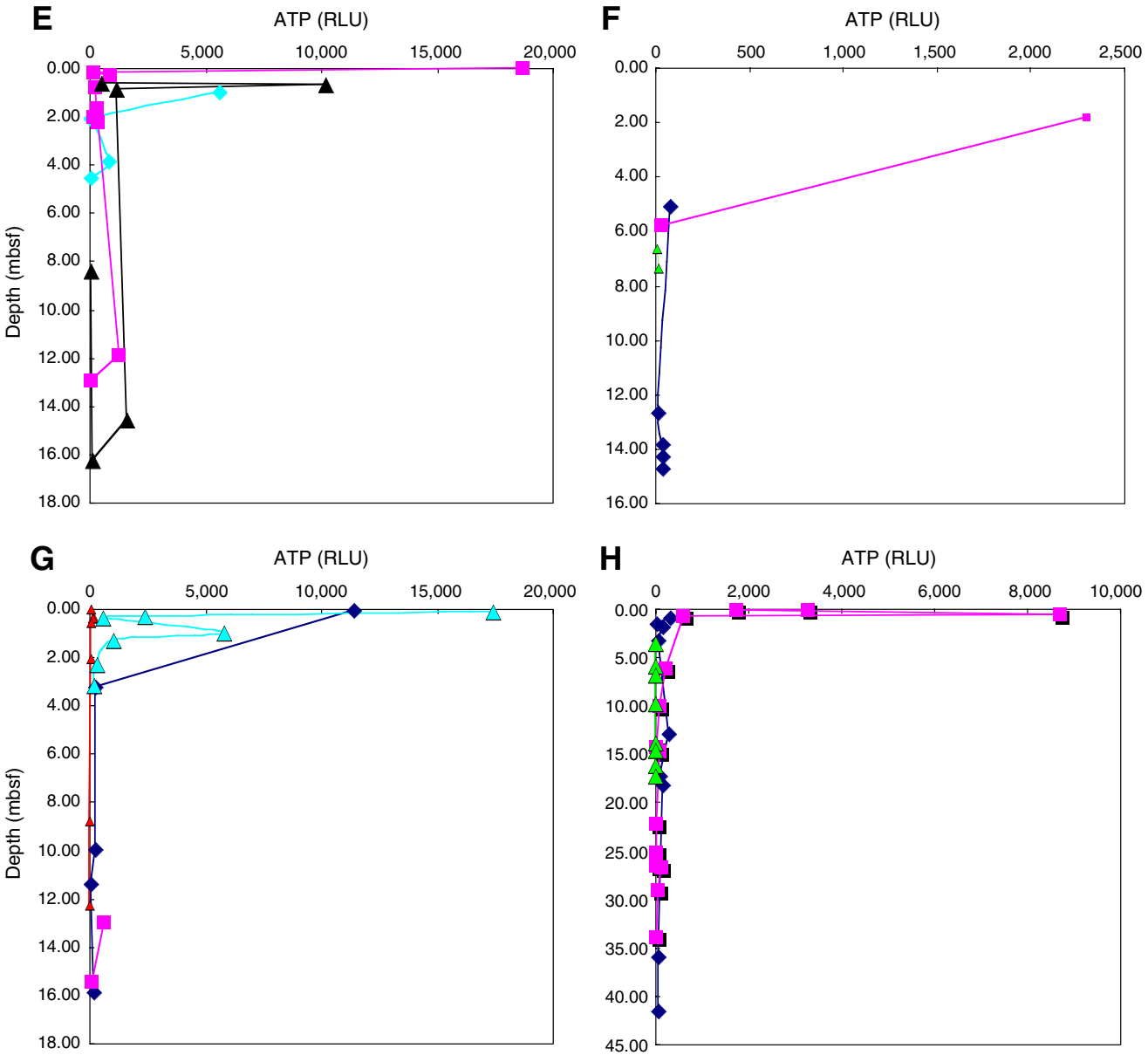

Figure F2 (continued). I. Holes M0015A (dark blue), M0016B (red), and M0017A (light blue). **J.** Hole M0015B. The very high activity near the surface was the sample with the blue/purple biofilm. **K.** Hole M0018A.

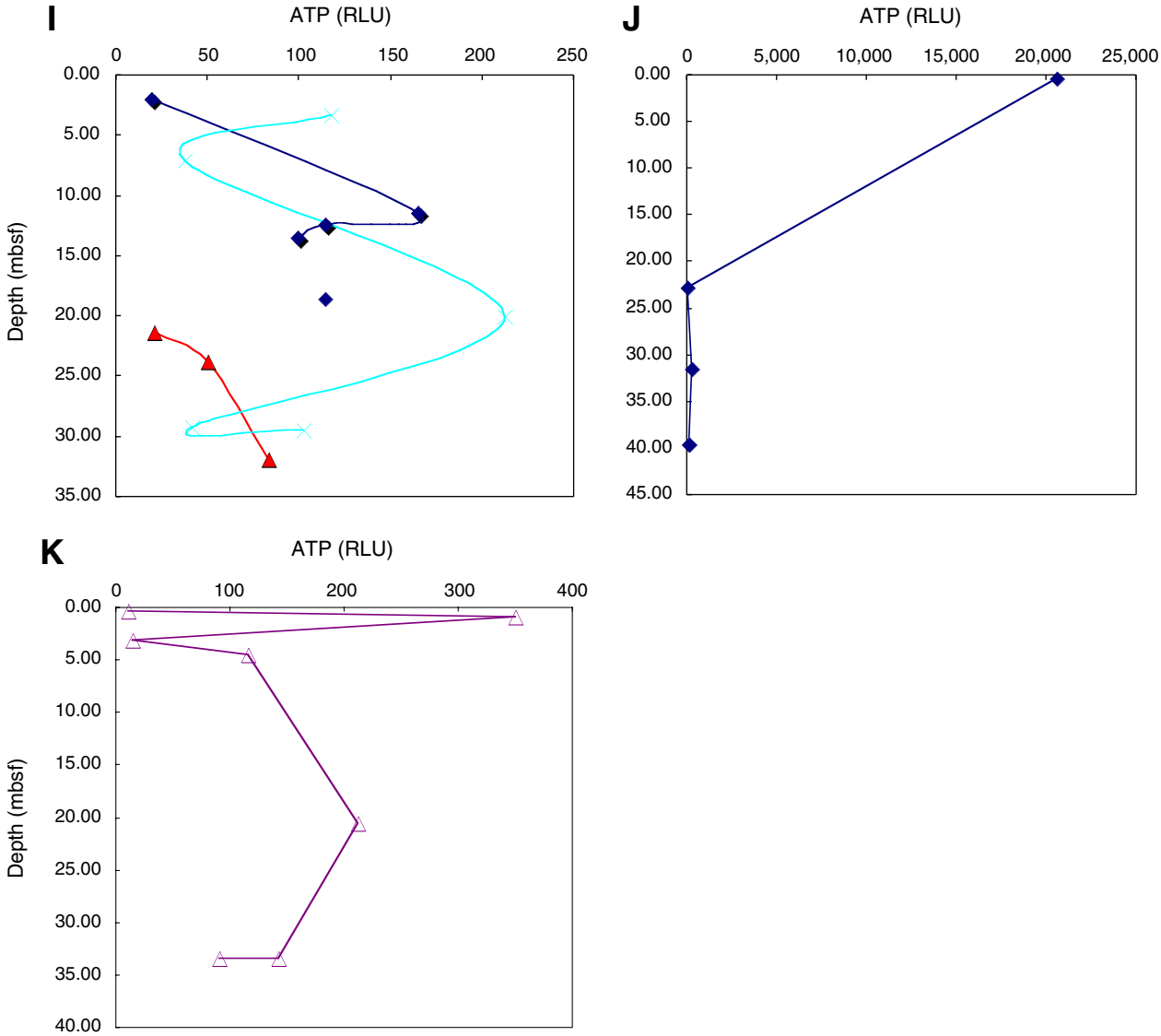

Figure F3. DAPI-stained samples under fluorescence microscopy. **A.** Eukaryotic organisms (e.g., sponges) and embedded clusters of microbes (blue dots) (Hole M0020A, 0.41 mbsf). **B.** Biofilm building microbial associations (blue dots) (Hole M0020A, 4.51 mbsf). Individual cells are small (diameter = ~0.4 µm). **C.** Biofilm building microbial associations (blue dots) (Hole M0020A, 15.49 mbsf). Individual cells are small (diameter = ~0.4 µm). **D.** Seven-cell aggregates of coccoid microbes (blue dots) (Hole M0023A, 0.12 mbsf). Individual cells are small (diameter = ~0.35 µm). Cells were abundant in this sample. (Continued on next two pages.)

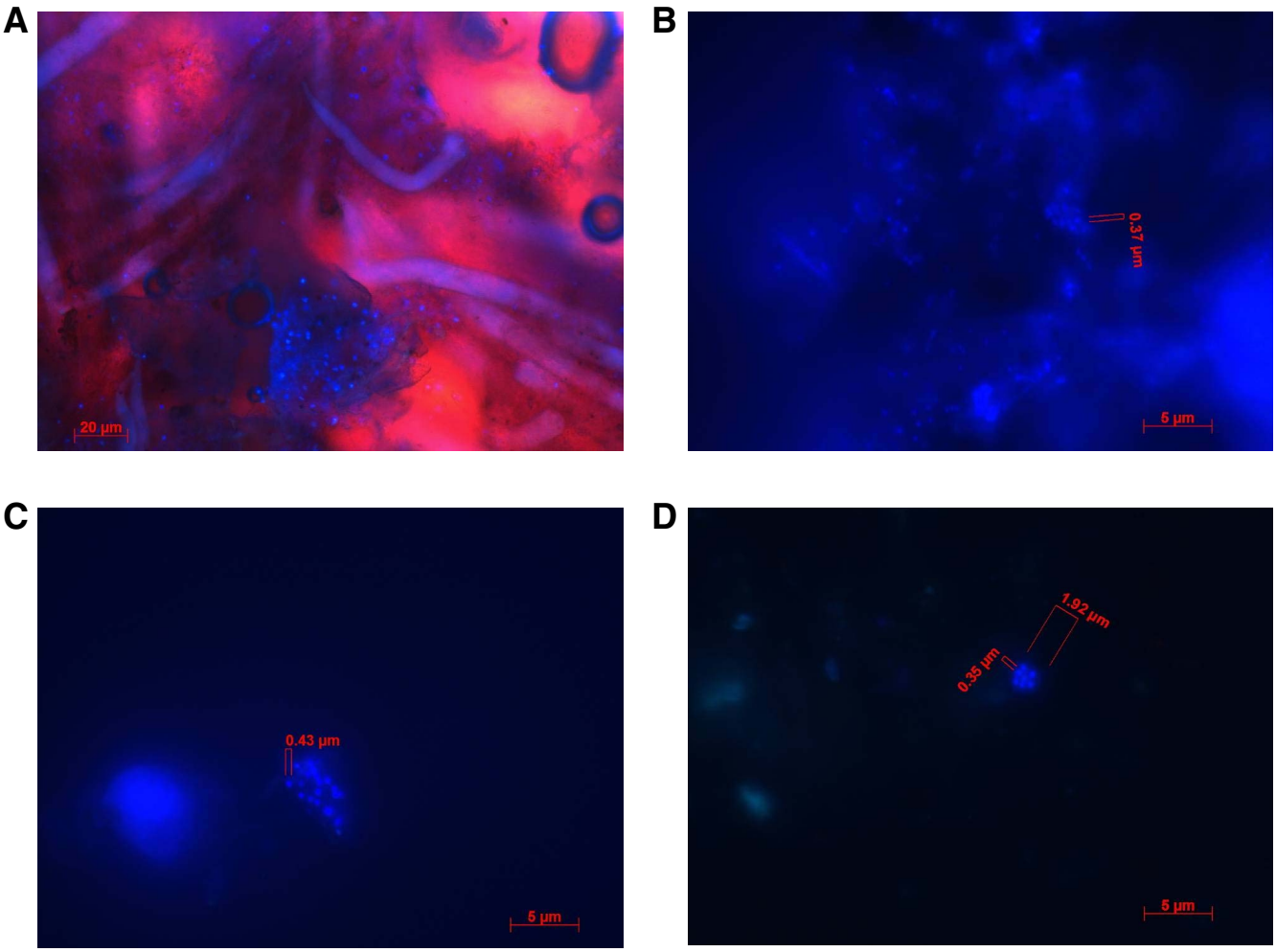

Figure F3 (continued). E. Cluster of small coccid cells (diameter = ~0.4 µm) (Hole M0023A, 0.12 mbsf). Cells were abundant in this sample. **F.** Cluster of small coccid cells (diameter = ~0.4 µm) (Hole M0021B, 0.61 mbsf). Cell density in this sample was low. **G.** Clusters of small coccid cells and a larger organism, probably a coral polyp larvae (Hole M0021B, 0.61 mbsf). **H.** Dividing cells, which means that they are actively growing (Hole M0021B, 11.88 mbsf). Overall cell density was low.

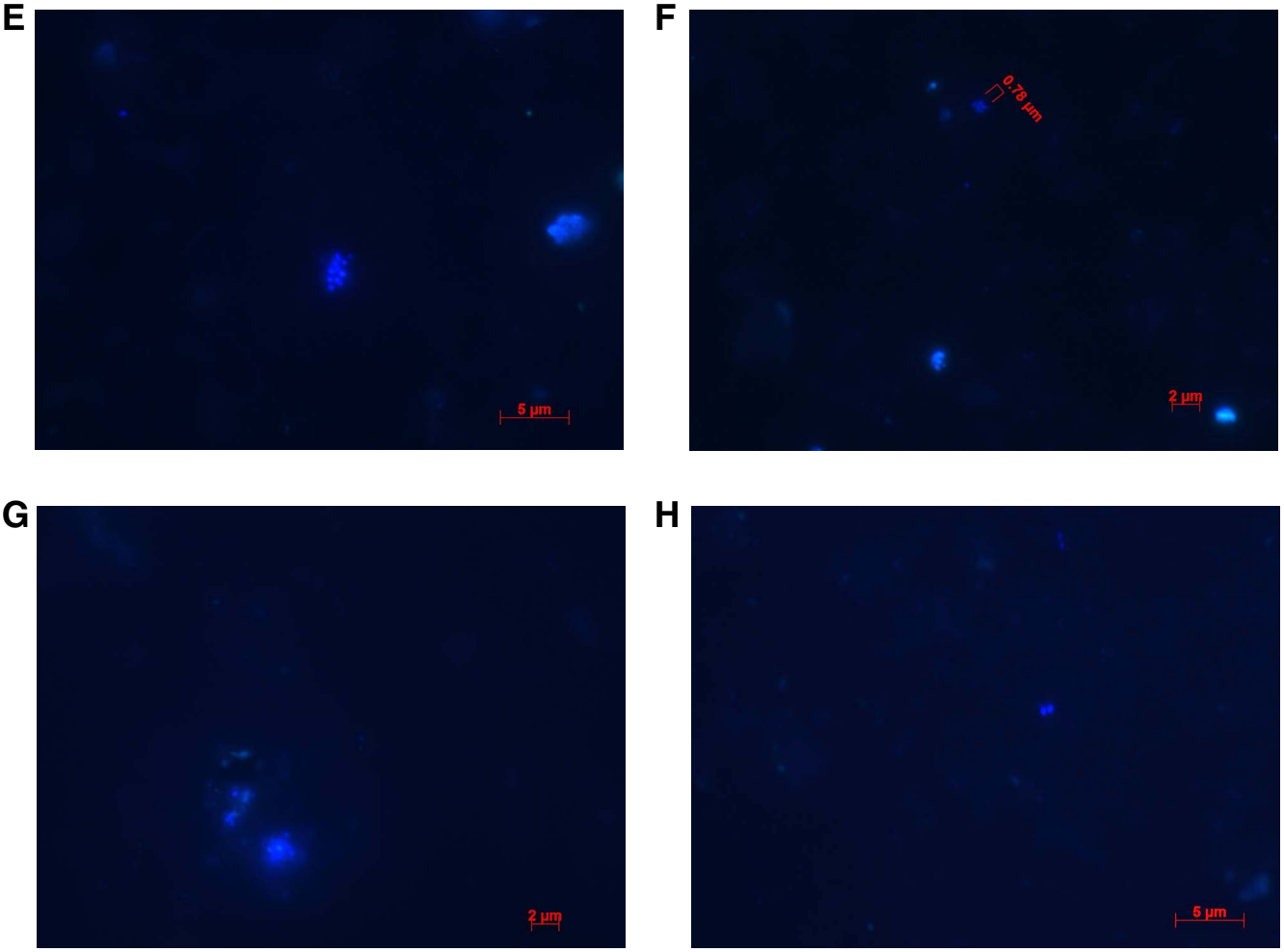

Figure F3 (continued). I. Chains of cells (Hole M0007C, 5.88 mbsf). Cells were common in this sample. **J.** Rather high diversity of cell morphologies (Hole M0007C, 5.88 mbsf). Cells were common in this sample. **K.** Blue/purple biofilm with carbonate mineral inside (Hole M0015B, 0.47 mbsf). **L.** High abundance of cells in a biofilm (Hole M0015B, 0.47 mbsf). **M.** Same as L, in a higher magnification, showing colonies of cells (~1.4 µm) in a biofilm.

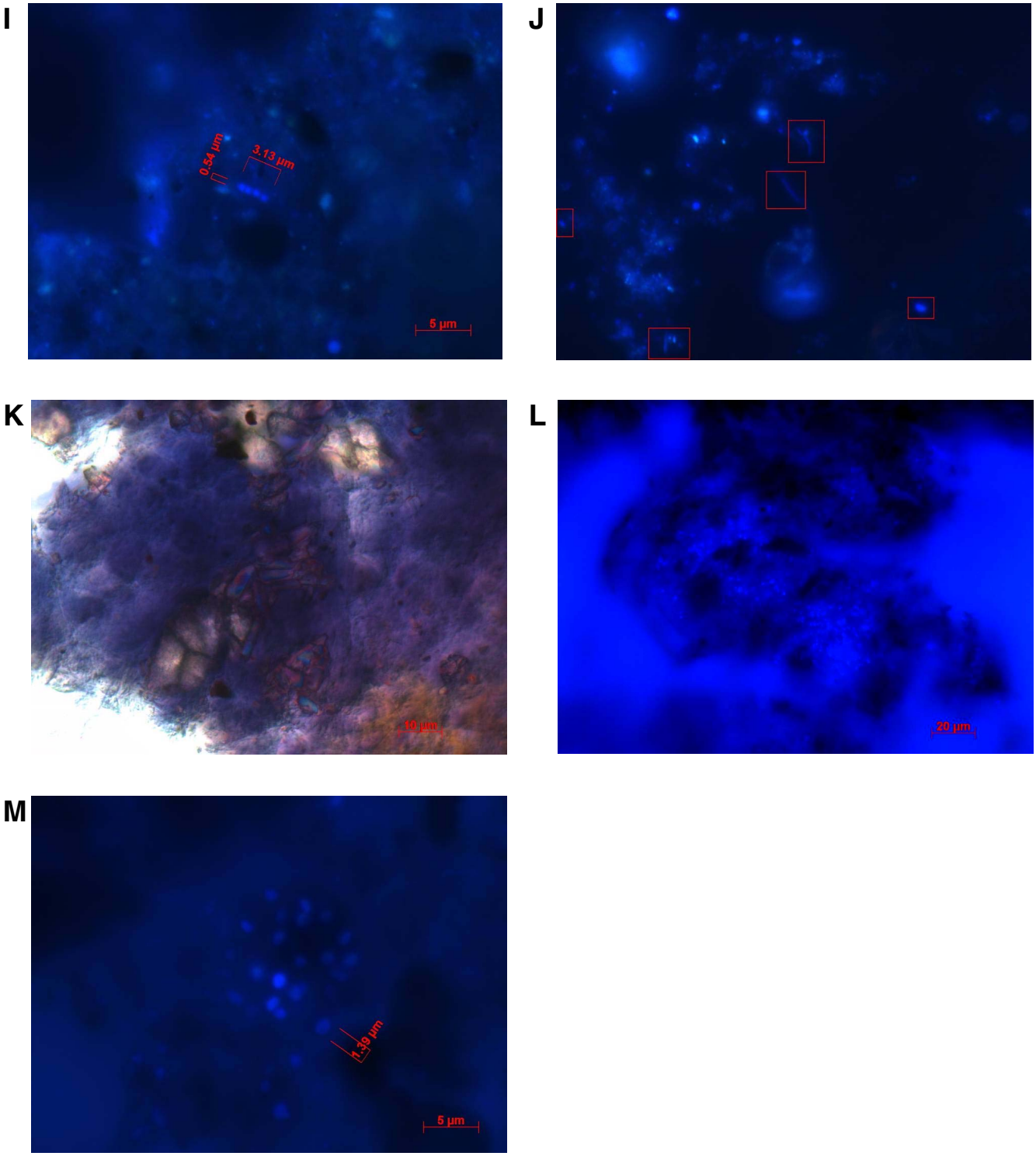

Figure F4. Glutaraldehyde-fixed samples. **A.** Surface of microbialites (magnification = 13,100-fold) (Hole M0020A, 4.51 mbsf). **B.** Surface of microbialites with pyrite crystals (magnification = 9,550-fold) (Hole M0023A, 0.12 mbsf). **C.** EPS-covered surface of microbialites with attached polyp larvae and possibly one coccoid bacterium in the foreground (magnification = 32,700-fold) (Hole M0024A, 11.88 mbsf). **D.** Surface of microbialites with some fiber type minerals, most probably clay minerals (magnification = 30,000-fold) (Hole M0007C, 5.88 mbsf). **E.** Surface of blue biofilm covered with a network of desiccated EPS minerals, presumably calcium carbonate and microorganisms (magnification = 19,200-fold) (Hole M0015B, 0.47 mbsf).

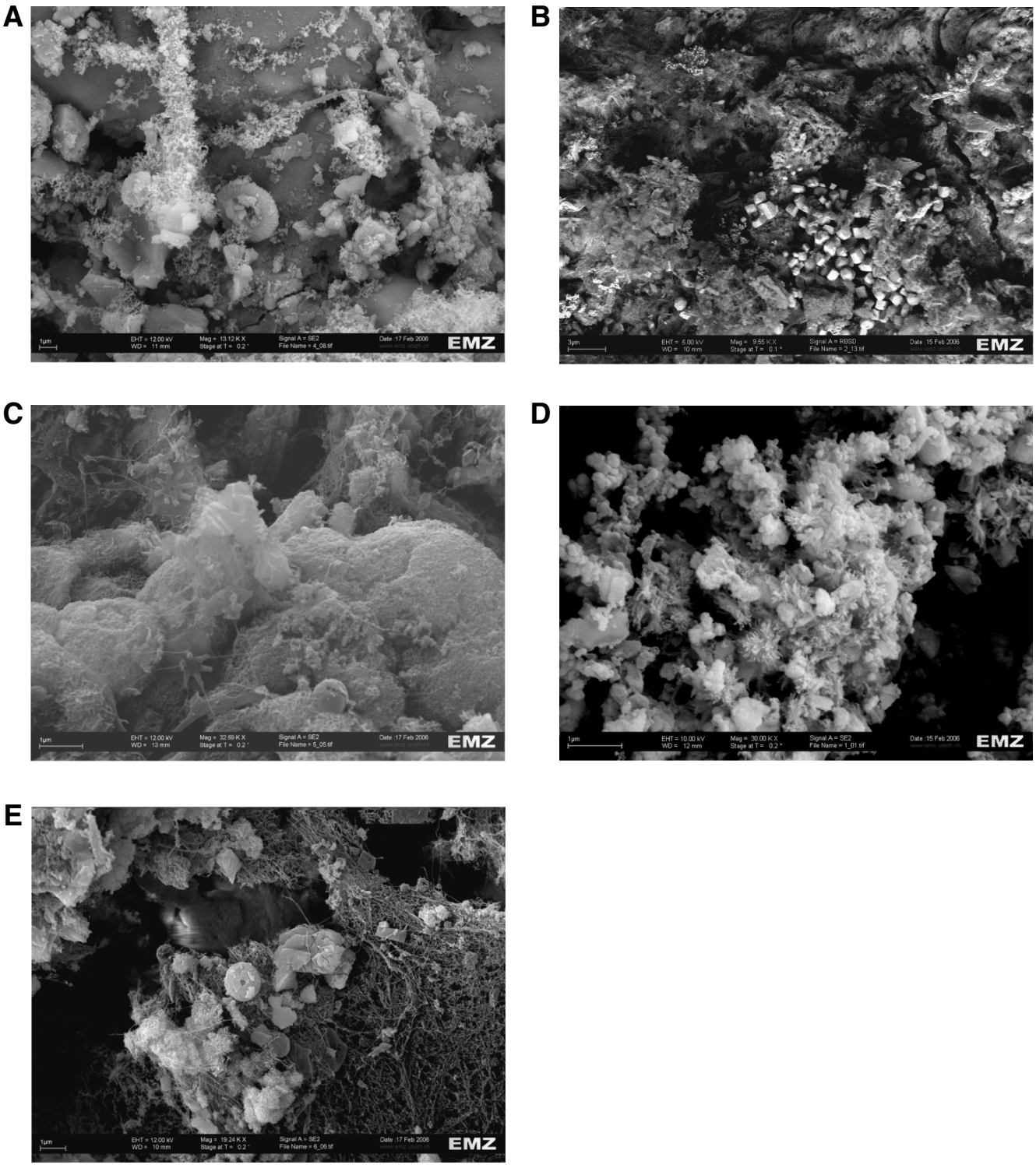

Figure F5. Cultures of microorganisms. Different colors originate from different pigmentation by carotenoids.

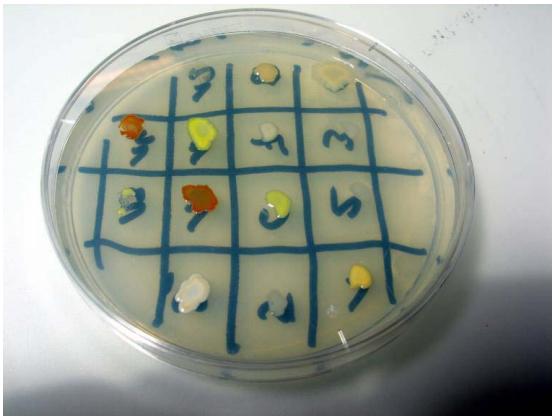

Figure F6. Light microscopy of pure cultures obtained during Expedition 310 (phase contrast; magnification = ~1000×). Letters correspond to Table T2. **A.** Interval 310-M0009D-5R-1, 1 cm. **B.** Interval 310-M0015B-2R-1, 0 cm. **C.** Interval 310-M0009D-5R-1, 1 cm. **D.** Interval 310-M0020A-6R-1, 12 cm. **E.** Interval 310-M0024A-1R-1, 68 cm. **F.** Interval 310-M0007C-3R-CC, 38 cm. **G.** Interval 310-M0020A-6R-1, 12 cm. **H.** Interval 310-M0005E-1M-1, 33 cm. **I.** Interval 310-M0024A-1R-1, 68 cm. **J.** Interval 310-M0016A-7R-1, 5 cm.

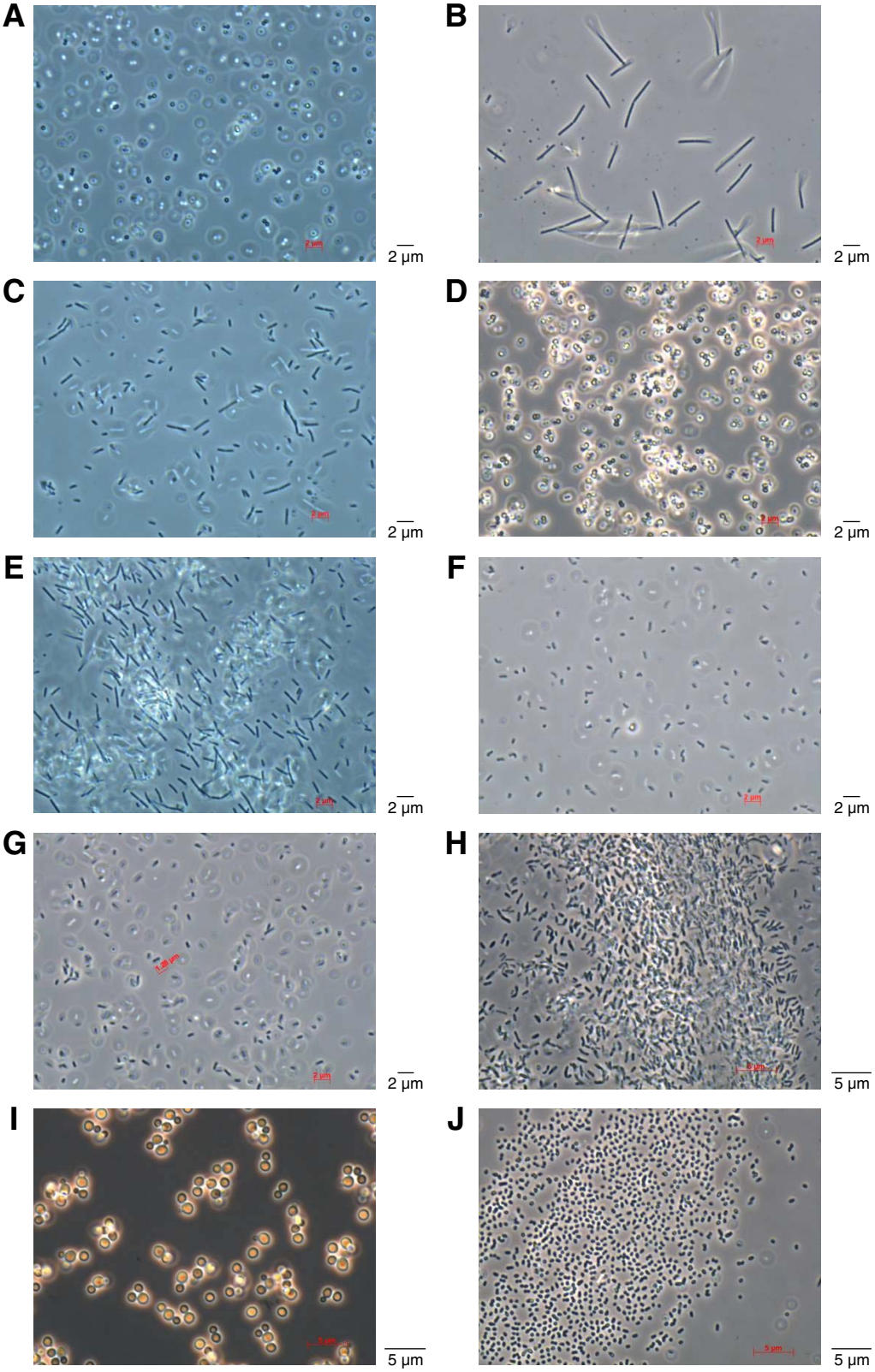

Table T1. Exoenzyme activity.

Hole	Depth (mbsf)	Exoenzyme (nmol/[g·h])		
		β-Glucosidase	Alkaline phosphatase	Aminopeptidase
M0020A	4.05	0	21.4	0
M0020A	4.51	0	79.9	2.4
M0025A	0.70	1.6	139.4	2.3
M0009D	3.64	0	255.6	0
M0005E	0.12	6.33	261.5	12.3
M0005E	0.33	68.5	65.02	9.7
M0005E	1.33	0	90.9	3.33
M0007B	0.12	1.75	156.3	7.69
M0007B	0.22	0	22.5	2.7
M0007B	0.75	0	13.56	0.37
M0007B	6.28	3.88	0	1.93
M0007C	5.88	0	12.7	4.38
M0015A	29.02	1.23	6.75	3.52

Table T2. Pure cultures of microorganisms obtained during Expedition 310.

Slide	Hole, core, section, interval (cm)	Cell morphology
	310-	
A	M0009D-5R-1, 1	Small coccoid cells, often in pairs
B	M0015B-2R-1, 0	Very long rods
C	M0009D-5R-1, 1	Rods with different lengths
D	M0020A-6R-1, 12	Irregular coccoid cells, forming aggregates
E	M0024A-1R-1, 68	Long rods
F	M0007C-3R-CC, 38	Short curved rods
G	M0020A-6R-1, 12	Short rods
H	M0005E-1M-1, 33	Short curved rods
I	M0024A-1R-1, 68	Large round cells
J	M0016A-7R-1, 5	Small coccoid to oval cells

Note: Slide letters refer to parts of Figure F6.