

**Survey of Shell-boring Microorganisms Across a
Depth Gradient at Point Caution, on San Juan Island, WA**

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ZooBot Research Apprenticeship

Spring 2011

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Keywords: euendoliths, microborers, coral, mollusk, bioerosion, algae, cyanobacteria, fungi, *Ostreobium*, *Leptolyngbya*, *Pharcidia*, *Phaeophila*, *Hyella*

Abstract

Although they are integral to many marine ecosystems, relatively little is known about euendolithic (shell-boring) organisms outside the tropics. Here, I present a short survey of euendoliths inhabiting a site on the Pacific coast of North America. Shells were collected from sites in the intertidal and subtidal zone off Point Caution, on San Juan Island, WA. Casts of boreholes were photographed with a scanning electron microscope, and their morphology and diameter was matched to previously isolated cultures. Several types of green algae, as well as cyanobacteria and fungi, were found in the intertidal shells. The 11 meter deep subtidal site was dominated by the green alga *Ostreobium*, and the 23 meter site by the cyanobacteria *Leptolyngbya*. Only heavily eroded tracings were found at the 30 meter deep site.

Introduction

Shell-boring organisms, or euendoliths, live inside CaCO_3 structures. They burrow through rock, as well as the calcareous skeletons of corals, mollusks, barnacles, and other marine invertebrates. This lifestyle has evolved separately in several different lineages of algae, as well as in cyanobacteria and fungi (Golubic *et al.* 2005, Matthes-Sears *et al.* 1999).

Euendoliths are of interest for three primary reasons. First, they manage the creation and redistribution of large amounts of biomass. As they burrow, they actively hollow out spaces in their substrate, releasing sediment into the water and contributing to erosion

(Tribollet 2008). They are often the first to colonize a newly-exposed surface; fish and other grazing animals follow, creating a positive feedback loop and encouraging further erosion (Chazottes *et al.* 1995, Gutiérrez *et al.* 2003). Because shells provide shelter and raw materials for many different species, overall ecosystem diversity may suffer if animals that produce them are destroyed (Gutiérrez *et al.* 2003). But in some reefs, euendoliths are also major producers of primary biomass (Tribollet *et al.* 2006). Therefore, understanding the relative importance of euendoliths' various roles is crucial to understanding their effect on the ecosystem.

Second, fossils of boring microorganisms can be used to map the geography of ancient bodies of water. In the upper photic zone, their fossil record is dominated by heterotrophic algae and cyanobacteria. As depth increases and available light decreases, heterotrophic fungi come to dominate. This stratification can be used to estimate the depth of ocean basins, where sedimentation is not informative (Golubic *et al.* 2005). Along with their abundant fossil record and evolutionarily conserved morphology, it makes euendoliths ideal for mapping ancient oceans.

Finally, boring microorganisms influence the global carbon cycle. Erosion of calcium carbonate (CO_3^{2-}) raises the concentration of CO_3^{2-} ions in seawater; some of this dissolved carbon eventually makes its way to the atmosphere and accumulates as gaseous carbon dioxide (CO_2). Current research has focused on the role of euendolithic microflora in bioerosion of coral reefs, and on how human activities may change the rate of erosion. The chlorophyte alga *Ostreobium* was found to be responsible for the majority of microbioerosion in tropical waters off the Great Barrier Reef, followed by the cyanobacteria *Plectonema*—also known as *Leptolyngbya*—and fungi (Tribollet 2008).

Microborers also penetrated deeper into their substrates in oligotrophic, offshore reefs than they did in eutrophic, inshore ones; this suggests that high grazing pressure and low sedimentation rates favor more extensive erosion. Other factors are also important: a balance exists between shell-boring autotrophs, which are limited by the available carbon, and heterotrophic fungi, which are limited by nitrogen and phosphorus (Carreiro-Silva *et al.* 2009). Pollution of the water with inorganic nutrients causes algae and cyanobacteria to flourish, while pollution with organic matter causes fungal hyphae to proliferate and decrease the available light. To understand whether pollution with organic carbon or inorganic nutrients is more likely to bring about climate change, the relative contributions to bioerosion by these two groups must be studied in more detail.

Although shell-boring microorganisms clearly play an important role in marine ecosystems and in the global carbon cycle, little is known about their composition and abundance outside the tropics. A survey of species inhabiting the Northeast Pacific would help give a broader context, both for the impact of human activities and for the interpretation of fossil data (Chazottes *et al.* 1995). In this project, I describe prevalent species found on San Juan Island, WA, focusing on variation in species composition with water depth.

Methods

Mollusk shells were collected off Point Caution at 11, 23, and 30 m depths on May 10, 2011. During low tide on May 18 (-0.8 m), additional shells were picked haphazardly from the lower intertidal. Shells were examined under a dissecting microscope for signs

of microborers, and one or two promising ones from each site were chosen for further study. As a reference, I examined shell fragments containing monocultures of likely algal species provided by Dr. Charles O’Kelly, Friday Harbor Laboratories, as well as images from prior studies (O’Kelly, unpublished).

Shells chosen from Point Caution were broken by hand into fragments. Three to four roughly 1cm² fragments were prepared for electron microscopy from each shell. The culture fragments were already of similar size, so were processed intact.

I prepared resin casts from each shell (Fig. 1) using a variation on the method developed by Golubric *et al.* in 1970. Shell fragments were covered in 1% formalin for 20+ min to stop growth. Then they were dehydrated in a series of acetone solutions: 25%, 50%, 75%, and 100%. They were left for at least 30 min at each concentration, and the 100% acetone was replaced after 15 min. Fragments were transferred to EMBED 812 embedding resin, and left at room temperature for 24 h. This allowed the resin to infiltrate the microscopic boreholes, as well as into the cytoplasm of any organisms they contained. Finally, a second batch of resin was prepared—this time with an accelerant to speed hardening. Fragments were dropped in the drying resin and baked at 60°C for 24 h to create solid resin blocks.

Square sections were cut from each block with a Dremel tool. Embedded shell fragments were dissolved with hydrochloric acid to expose the resin molds. Sections corresponding to the inner surfaces of the shells were glued to metal stubs for use in the scanning electron microscope (SEM). Inner surfaces were chosen because they were less likely to

have accumulated boreholes during the animals' lives, and therefore more likely to represent the depth at which they were found.

Only conductive objects can be seen with an SEM, so I used a sputter coater to shower them with a thin layer of gold/palladium. Then each was examined and photographed. The stubs were saved, along with representative pictures from each shell.

Several types of euendoliths were identified visually by comparison of their boreholes with previous SEM pictures (Golubic *et al.* 2005; O'Kelly, unpublished work). Average tunnel diameter was also used to distinguish among morphologically similar casts.

Results

Tracings were identified from a variety of euendolithic organisms (Table 1). Species composition was different at each depth studied, with the majority of borings found in the intertidal and 11 m subtidal sites.

Borings in shells collected from the intertidal zone (Fig. 2 - 3) can largely be attributed to the cyanobacteria *Hyella* and *Leptolyngbya*, and the green alga *Phaeophila*. However, very fine filaments were also common. Their diameters were significantly smaller than *Leptolyngbya* ($P < .001$), and they had true branches. This size and morphology are consistent with fungal hyphae (Golubic *et al.* 2005)—most likely *Pharcidia balani*.

Ostreobium, a green alga (Fig. 4), was the only micro-borer found in shells from a depth of 11 m. In addition to the expected branching pattern, large tunnels running normal to the surface of the shell were also observed; their shape was reminiscent of *Hyella*, but

continuity with the smaller tunnels suggests that they represent sporangia rather than a separate group. They were rarely found without surrounding *Ostreobium*. Some of these large tunnels were heavily eroded.

Leptolyngbya was found at the 23 m site (Fig. 5). Its tunnels were identified primarily by size. At about two microns in diameter, they were not significantly different from cultured strains F146, F147, and F148 (Dunn's Multiple Comparison test; $P = 0.166$). They were also distinguished from fungal hyphae in that they lacked true branches. The other prominent feature of the shells from this site was an unknown type of large tunnel—possibly animal boreholes. Their shape was similar to *Hyella*, but their diameters were significantly larger (Dunn's Multiple Comparison test; $P < 0.001$).

There was no evidence of recent microborings in shells from the 30 m deep site. Large networks of boreholes were visible (Fig. 6), but they had eroded too thoroughly for reliable classification.

Discussion

Light availability is clearly a major factor in determining the species composition of euendolithic communities: shells from the intertidal and shallow subtidal (11 m) sites were covered in photosynthetic algae and cyanobacteria, while shells from the deeper sites (23 and 30 m) showed relatively few signs of life. The networks of eroded tunnels observed at 30 m were likely formed by photosynthetic microflora close to the surface, which were then starved of light after their shells were relocated.

Other factors, such as temperature and nutrient availability, do not appear to be as important. The same broad groups of euendoliths described in my study—chlorophyte algae, cyanobacteria, and fungi—have been identified as key players in other euendolithic habitats. *Ostreobium* and *Leptolyngbya*, specifically, cause the majority of microbioerosion on the Great Barrier Reef (Tribollet 2008). These similarities suggest that euendolithic ecology may be relatively uniform across the world.

These are interesting preliminary results, but their predictive power is limited by small sample size and pseudoreplication; I measured the diameters of hundreds of tunnels, but only a few shells and a few genetically distinct “individuals” per group. Larger and more comprehensive studies are needed to determine whether my findings are generally applicable.

In conclusion, the euendolithic community at Point Caution is generally similar to those previously described in the tropics. As in the Great Barrier Reef, *Ostreobium* and *Leptolyngbya*, along with fungi, were the most prevalent groups. This suggests that the factors influencing their growth rates, while relatively simple, are also of great significance to the global ecosystem.

Acknowledgements

I would like to thank Aaron Galloway for collecting shells during his dive, the Summers lab for providing a workspace and access to the scanning electron microscope, as well as Charles O’Kelly, who gave me guidance, supplies, and algal cultures. Finally, I would like to thank the Mary Gates foundation for their support.

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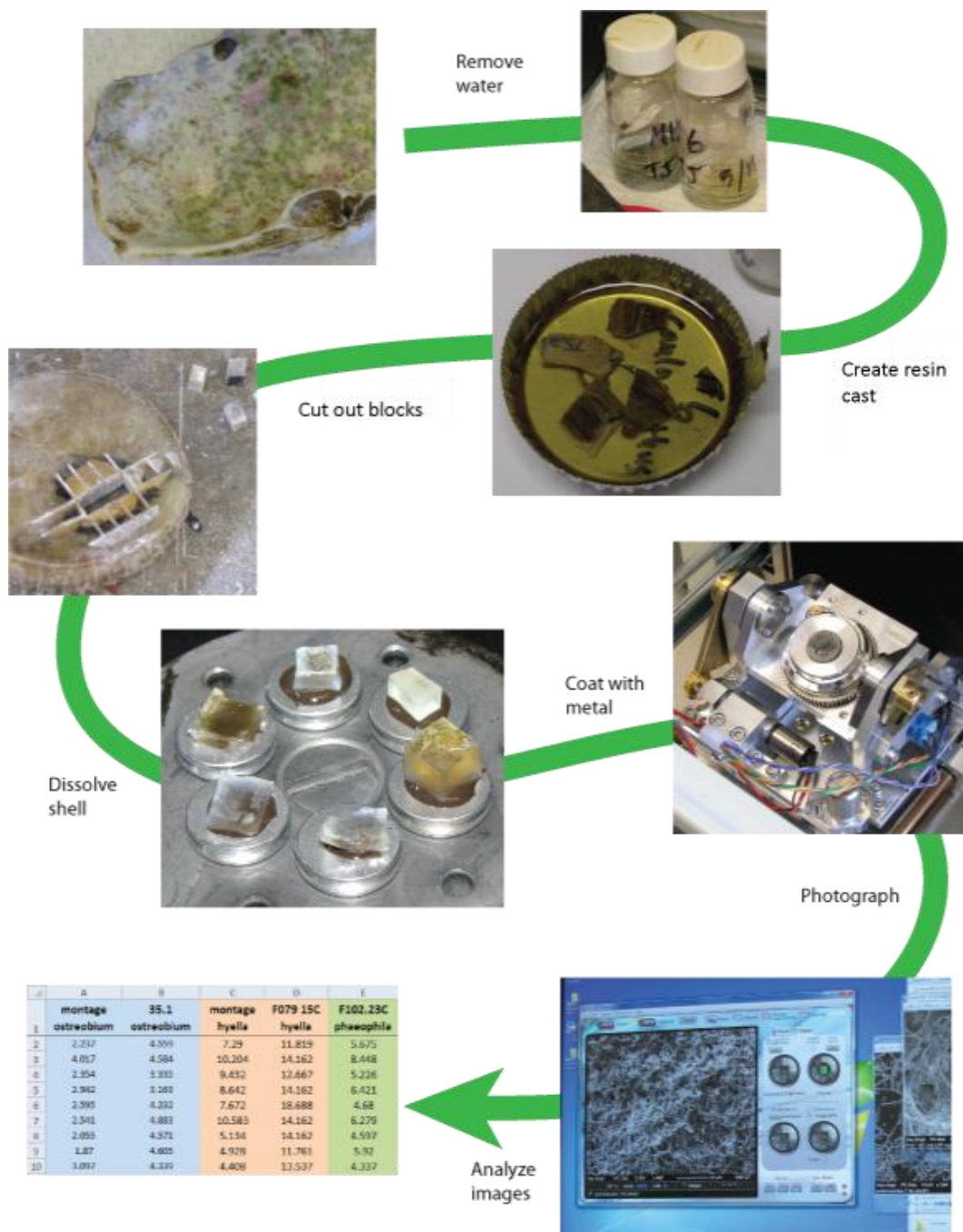


Figure 1. My workflow for examining microborings under the SEM.

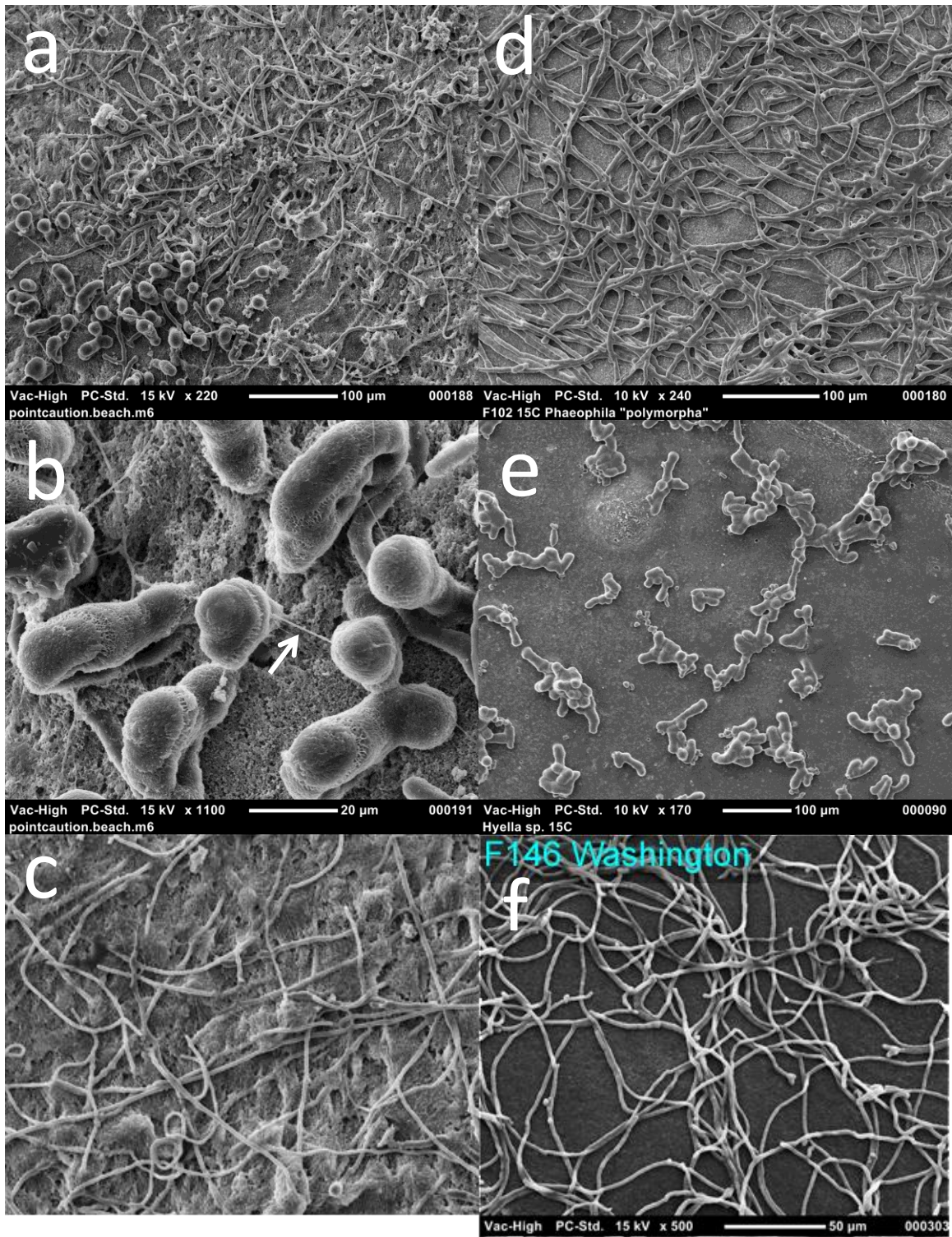


Figure 2. SEM images of microboring casts. **a, b, c.** Boreholes inside a shell from the intertidal zone at Point Caution. From top to bottom: *Phaeophila* and *Hyella*, *Hyella* draped with fungal hyphae (arrow), and *Leptolyngbya*. **d, e, f.** Boreholes produced by euendoliths cultured in shell fragments. From top to bottom: *Phaeophila*, *Hyella*, and *Leptolyngbya*.

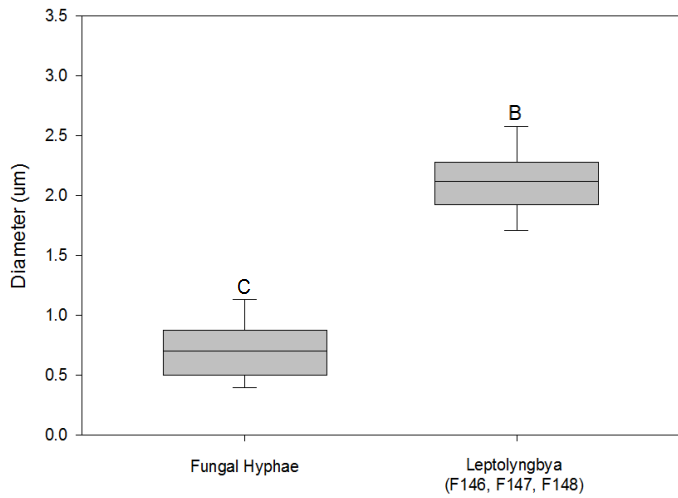
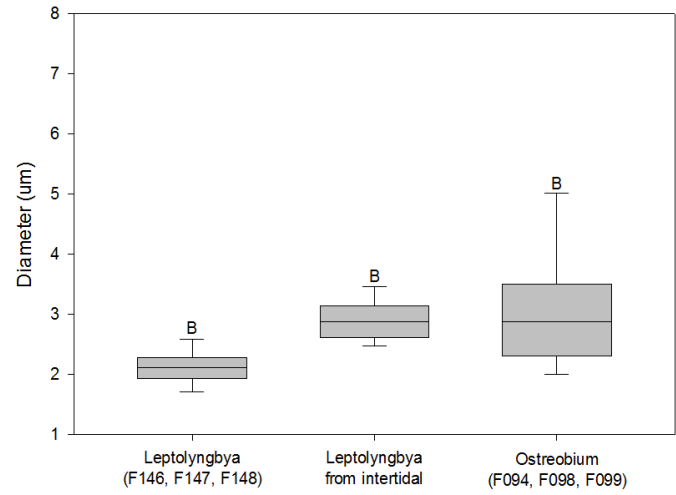
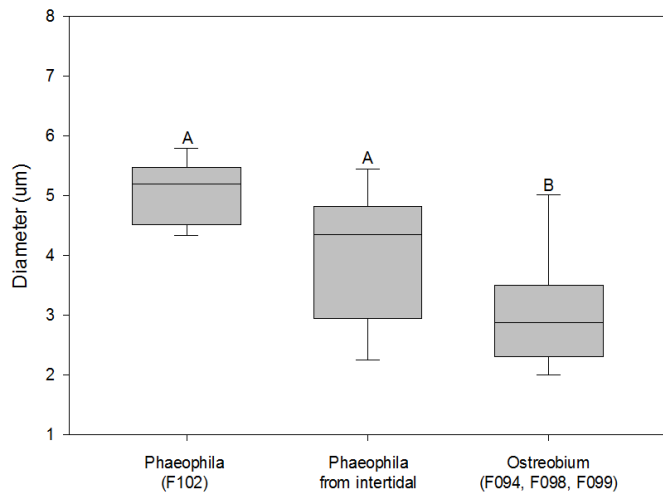


Figure 3. Average diameter of tunnels produced by different microborers found in the intertidal zone at Point Caution. Letters indicate groups that do not differ significantly ($P > 0.05$) according to Dunn's Multiple Comparison test.

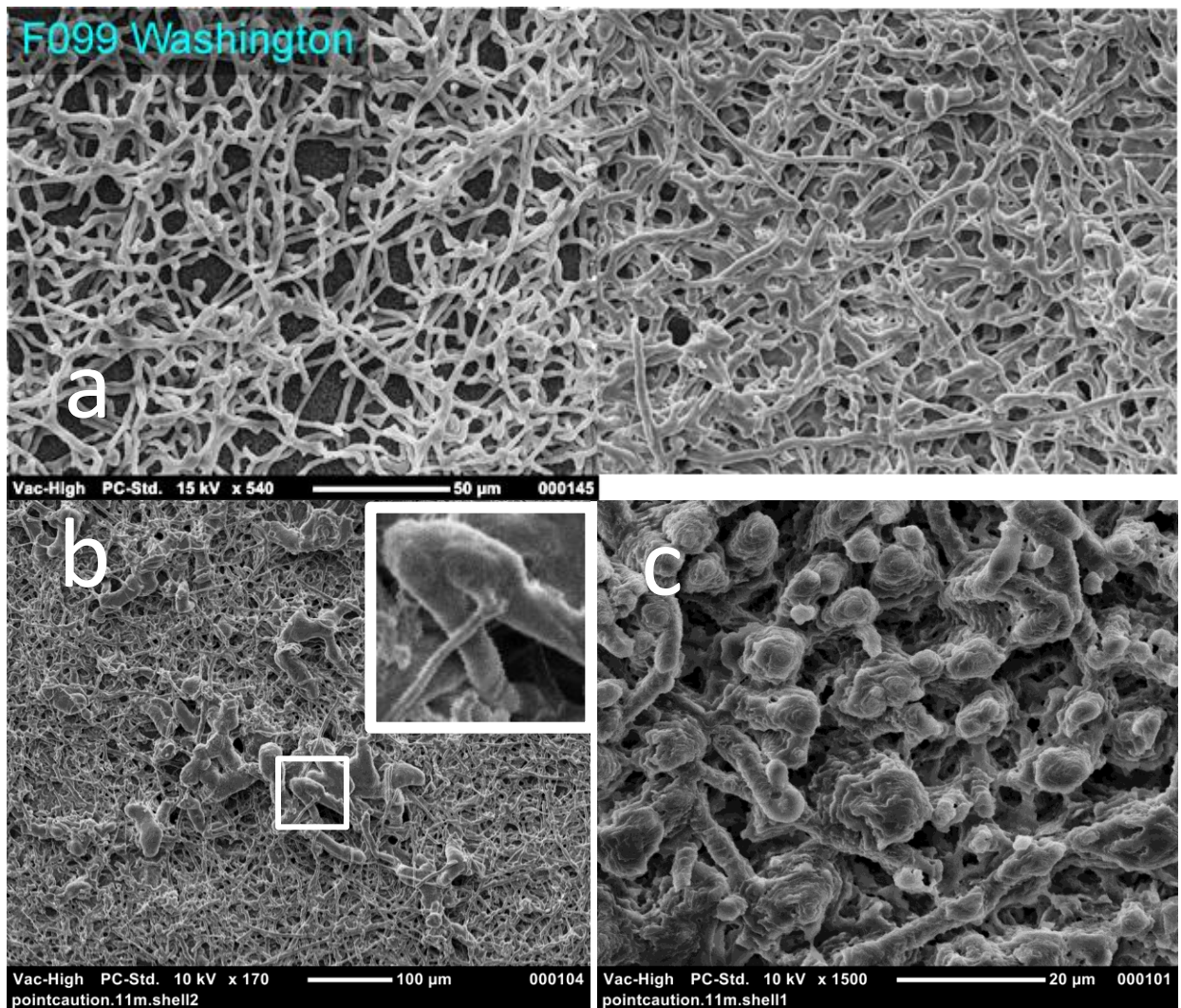


Figure 4. *Ostreobium* casts. **a.** Cultured strain (left), along with a similar specimen (right) from 11 meters deep off Point Caution. **b.** Junction between small and large boreholes. **c.** Partially eroded boreholes.

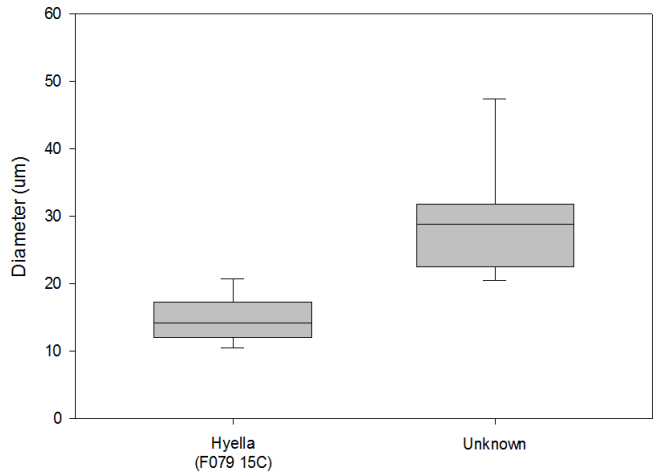
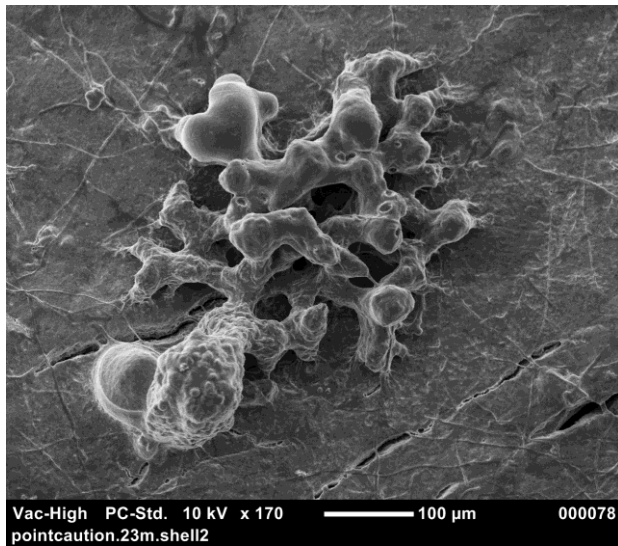


Figure 5. Unknown boreholes found at the 23 m Point Caution dive site, and a comparison of their diameter with cultured *Hyella*.

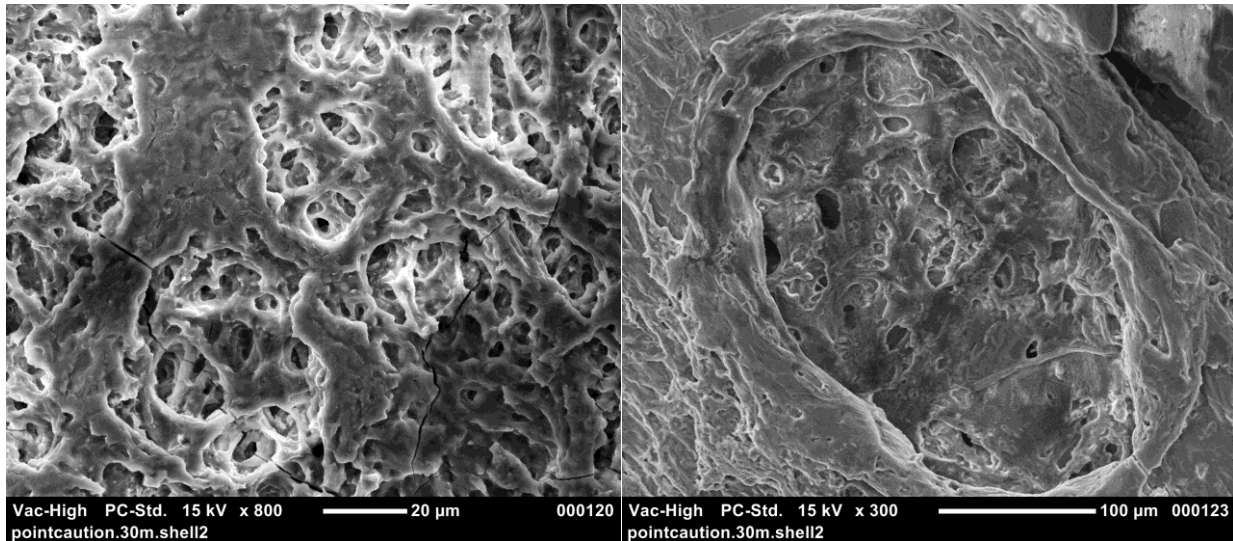


Figure 6. Ambiguous boreholes found at the 30 m Point Caution dive site.

Depth	Phaeophila	Ostreobium	Hyella	Leptolyngbya	Fungi	Unknown
0m	✓		✓	✓	✓	
11m		✓				
23m				✓		✓
30m						✓

Table 1. Summary: Zonation of shell-boring microorganisms at Point Caution.