Diversity of carbonate-boring algae: Morphology and molecules

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Abstract

Despite the name, carbonate-boring algae are in fact quite interesting. Contributing equivalent to 20% of CO_2 to the biosphere as human activities through their $CaCO_3$ dissolution, these algae have broad ecological impacts. Morphological analysis of five genera of carbonate-boring algae found at Argyle Lagoon, San Juan Island, WA, as well as analysis of 3-dimensional resin casts of the bore holes, showed that using morphology to study diversity tells an incomplete story. Phylogenetic analysis of laboratory algal cultures of cool-temperate carbonate borers revealed a strong genetic difference between subtidal and intertidal species studied, indicating that these algae possess at least some degree of tidal height zonation.

Introduction

Carbonate boring algae play important roles in marine ecosystems as agents of bioerosion, primary producers for grazers like parrotfish, and habitat builders for chasmoendoliths (Golubic, *et al*. 1981). These algae inhabit a wide geographic and temperature range from tropical equatorial waters to the Antarctic (reviewed in Tribollet 2007). Boring traces created by these algae can be used for species identification, as each species leaves a distinctly shaped bore hole (Golubic 1969).

Although the mechanism of carbonate dissolution has not been thoroughly studied, a common method used by boring cyanobacteria is calcium uptake at the apical cell, transfer, and deposition away from the active boring, mediated by calcium-ATPase (Ramírez-Reinat and Garcia-Pichel 2011). Through this dissolution process, carbonate-borers are estimated to contribute as much CO_2 into the ocean to equal 20% of all CO_2

from human activities (Tribollet *et al.* 2006). Consequences of this dissolution are not thoroughly studied but have broad-reaching ecological implications for the marine environment.

The rates of carbonate dissolution are increased when inorganic nutrients like nitrogen and phosphorous are increased in euendolithic algae abundance, subsequently increasing the microbioerosion rates (Carreiro-Silva *et al.* 2009). Microbioerosion rates slow with time as the substrate becomes more densely colonized (Vogel *et al*. 2007). However, a positive feedback loop can begin with other macrocolonizing organisms like sponges that create more free surface available for microboring and thus more bioerosion (Carreiro-Silva *et al.* 2009). Euendolithic organisms like boring algae that actively dissolve the substrate weaken it overall (Ginsberg 1953).

Diversity of cyanobacterial euendoliths has likely been underestimated based on new genetic diversity being revealed with molecular techniques (Ramírez-Reinat and Garcia-Pichel 2011). *Ostreobium quekettii*, a common euendolith, is far more genotypically diverse than previously thought and is comprised of several clades (Gutner-Hoch and Fine 2011). Although diversity of carbonate boring algae from tropical regions has been assessed (Vogel, *et al*. 2007), few studies have evaluated temperate species. Furthermore, understanding the biodiversity of these micro-communities is essential for further understanding the ecology and contribution of individual species and taxonomic groups. This study seeks to assess and compare the diversity of shell-boring algae of a lagoon channel in the Pacific northwest of the United States using morphology. In addition, this study compares algal cultures grown at different temperatures and assesses genetic differences among strains.

Currently shell-boring algae samples from the Salish Sea have been randomly collected with no assessment of possible biogeography of these organisms. The Argyle Lagoon study compares diversity across tidal height. What is the diversity of carbonate-boring algae in a Salish Sea lagoon channel and does this diversity change with tidal height? Cultured algae from the similar habitat of Moss Landing, CA, were grown at differing temperatures. Are these California algae genetically different from the Salish Sea algae? Do they exhibit different morphologies under different temperatures?

Methods

Study Site and Sampling

Argyle Lagoon is located along the southeastern coast of San Juan Island, WA, USA (48.520138, -123.014675). The lagoon possesses a curious formation. A narrow channel feeds the lagoon, with water flowing rapidly in and out with the tides. A transect (**Fig. 1**) was created to investigate the effects of tidal height on the diversity of carbonate-boring algae. An elevation decrease from the lagoon side of the channel to the ocean side of the channel causes higher tidal height over the upper channel locations (close to the lagoon) than compared to the relatively low tidal height on the lower ocean side of the channel. The lower part of the channel, (LC) 48.521343,-123.014777, the middle of the channel, (MC) 48.521636,-123.014538, and the higher part of the channel (HC), 48.521698, -123.013951, create the transect.

Shell fragments of clams and barnacles with visible algae borings were selected randomly at each transect point by tossing a stick and collecting where the marked end landed. Shells were collected outwards concentrically until 5-10 suitable shell fragments were acquired. Collection occurred at low tide on June 20, 2012.

After any biofilm material was scraped off with a toothpick, shells were partially dissolved in 10% HCl to liberate the euendolithic algae. The extracted algae were then rinsed in distilled RO water and transferred to a microscope slide. A Nikon Eclipse E600 microscope with a QImaging Micropublisher 5.0 RTV camera and Photoshop and QCapture software were used for microphotography.

Shell fragments collected at the Argyle Lagoon transect site were also processed to make a cast of the bore holes for SEM imaging and analyzing. Shell fragments were rinsed with 1% formalin for at least 15 minutes to kill algae. Shells were soaked in 25%, 50%, 75%, and then 100% acetone for at least 30 minutes per concentration. Resin was mixed according to the kit from EMBed-812 (emsdiasum.com). The shells were immersed in resin and baked overnight. Once hardened, shells were cut with a diamond saw. Shell fragments were dissolved in 100% HCl and then rinsed in water and ethanol before being epoxy-glued to an SEM stub. Photos were taken using the SEM imaging software Start JCM 5000 and boring trace diameters measured with ImageJ software.

Cultured Algae

Cultured cyanobacteria isolated from intertidal barnacles and subtidal clam shells from Moss Landing, CA, were used to compare growth success at two temperatures, as well as to conduct genetic analysis. Four replicates of each algal clone line were placed in incubators: two in 15º C and two in 23º C in f/2- medium (Guillard and Ryther 1962). Algae were cultivated under a light irradiance ranging between 11.9-55.5μE/m^2/s. Additionally, an oyster shell was placed in one replicate of each clone at each temperature to assess boring behavior. Light microscopy photos of the algae were taken using a Nikon Eclipse E600 microscope and a QImaging Micropublisher 5.0 RTV camera.

In addition to morphology, genetic analysis was also performed. Cyanobacterial DNA was extracted using a MO BIO UltraClean® Plant DNA Isolation Kit (MO BIO Laboratories, Inc.). Two cyanobacterial primers, CYA106F and CYA781R (Nubel et al., 1997), were used for isolation and PCR amplification of a fragment of the 16s ribosomal RNA gene. Successful PCR projects were sent to GeneWiz (genewiz.com) for sequencing. Nucleotide sequences were aligned with Sequencher® software and phylogenetic analysis was performed with MEGA© software. Neighbor-joining gene trees were created with 500 bootstrap resamplings.

Results

Five different groups of carbonate-boring algae were found across the Argyle Lagoon tidal height transect. These algae and their corresponding average bore trace diameters and standard deviations are summarized in **Table 1**. Ichnotaxa were used for species identification from SEM photographs instead of the biological nomenclature because photos and measurements were of the trace, not of the algal cells. Except for *Cavernula pediculata*, all bore traces were of filamentous algae.

All of the cool-temperate California algae cultures grew well both in their native temperature of 15˚C and at the subtropical temperature of 23ºC. Three of the cyanobacteria cultures of the *Plectonema* type from Moss Landing, CA, exhibited chromatic adaptation between the two temperatures. Cultures grown at 15ºC remained green whereas when grown at 23ºC transformed into a dark reddish color.

Two phylogenetic trees were constructed from cool-temperate carbonate-borers of the *Plectonema/Leptolyngbya* and *Hyella* species. All carbonate-boring *Plectonema,* which possess similar morphologies, were grouped into essentially one distinct clade (**Fig. 2**). Contrarily, the *Hyella* species, which also possess very similar morphologies, had variable genetics (**Fig. 3**). The deep subtidal species were grouped together in one clade, and the intertidal species were grouped together in an entirely different clade. The shallow subtidal species were scattered across the gene tree, including being mixed in with both the deep subtidal and intertidal *Hyellas*.

Discussion

The five morphologies found in the Argyle Lagoon samples are likely a severe underestimate of the true biodiversity of this habitat. Previous biodiversity studies of carbonate-boring algae focused primarily on using morphology to distinguish taxa (Radtke and Golubic 2005). However, morphology possesses several limitations. Because little is known about varying morphology within species, meaning even a single species could possess several morphotypes across different environmental conditions and habitats ranges, it may be unreliable to continue characterizing and studying species based on visual identification alone. In this study, the samples collected from Argyle Lagoon could only be identified to genus. Although the ichnotaxa follow a different nomenclature and bore traces are specific to an algal species, the ichnotaxonomy is based off the algal taxonomy, which is currently not representative of the known diversity that genetic analysis has shed recent light on. Thus, although morphology is crucial to include in tandem with molecular techniques, it is apparent that it cannot stand alone.

The phylogenetic analysis performed on the *Hyella* types from temperate environments give evidence for zonation across tidal height. While shallow subtidal species are scattered throughout the tree, the intertidal species are grouped together and are entirely separate from the deeper subtidal species. Additionally, the carbonate-boring *Hyella* do not form a separate clade from other non-boring algae, whereas the carbonate-boring *Leptolyngbya/Plectonema* species do form a distinct clade. This may indicate that boring behavior evolved separately among these algae and that in general, carbonate-boring algae overall do not form a distinct clade.

Furthermore, the phylogenetic analysis reveals that morphology is not adequate as a sole method of identification. The *Hyella* and *Plectonema* types both had similar morphologies within each respective group, and while *Plectonema* formed a distinct clade, the *Hyella* group did not.

The chromatic adapation exhibited by three of the *Plectonema* strains is unusual since other subtidal populations of *Plectonema* do not show this phenomenon in culture. Phycoerythrin synthesis, which is correlated with chromatic adaptation, seems to be regulated by light quality (Tandeau de Marsac 1977). However, the wavelength of light used in this study was a constant white light, and so a possible explanation for this adaptation could be temperature-dependent but there currently is no data to support this.

Carbonate-boring algae have far reaching ecological implications as major primary producers in dead coral reefs, as well as significant contributors to oceanic CO_2 (Tribollet *et al*. 2006). However, research on these algae has been primarily on taxonomy and diversity and has only recently expanded to further ecological roles and responses. Due to the limitations of morphology, genetic analysis will likely be the focus of future taxonomic research on these algae.

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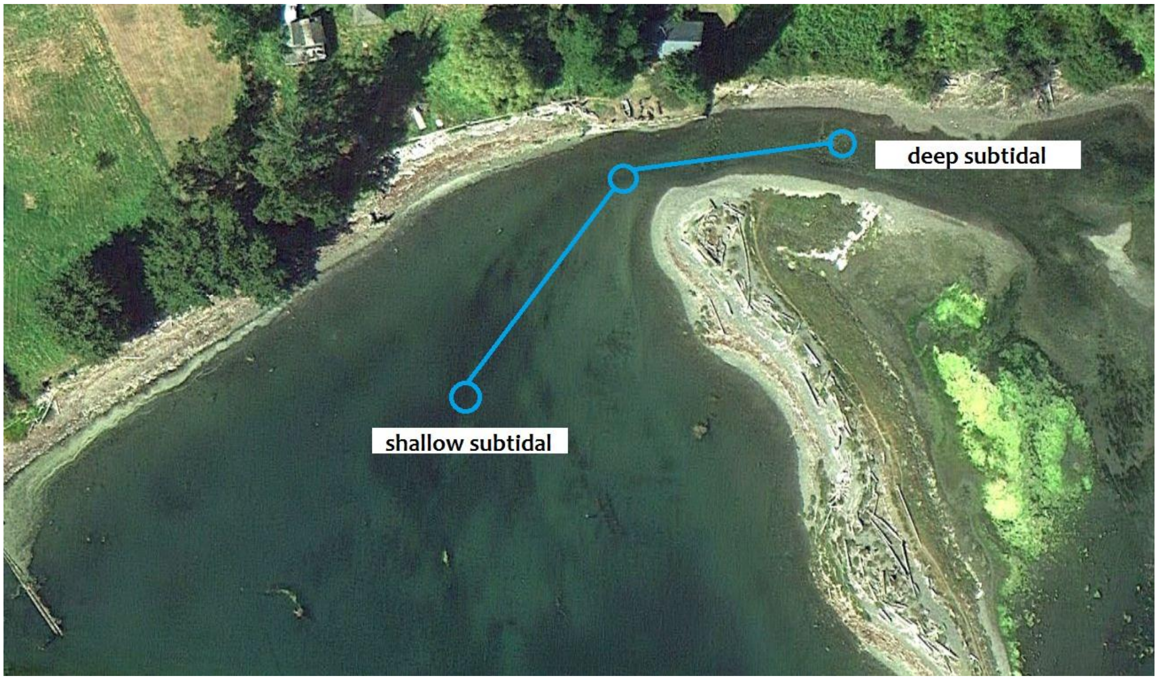

Figure 1. Argyle Lagoon sampling locations: blue marks the tidal height transect and purple marks the tidal emersion transect.

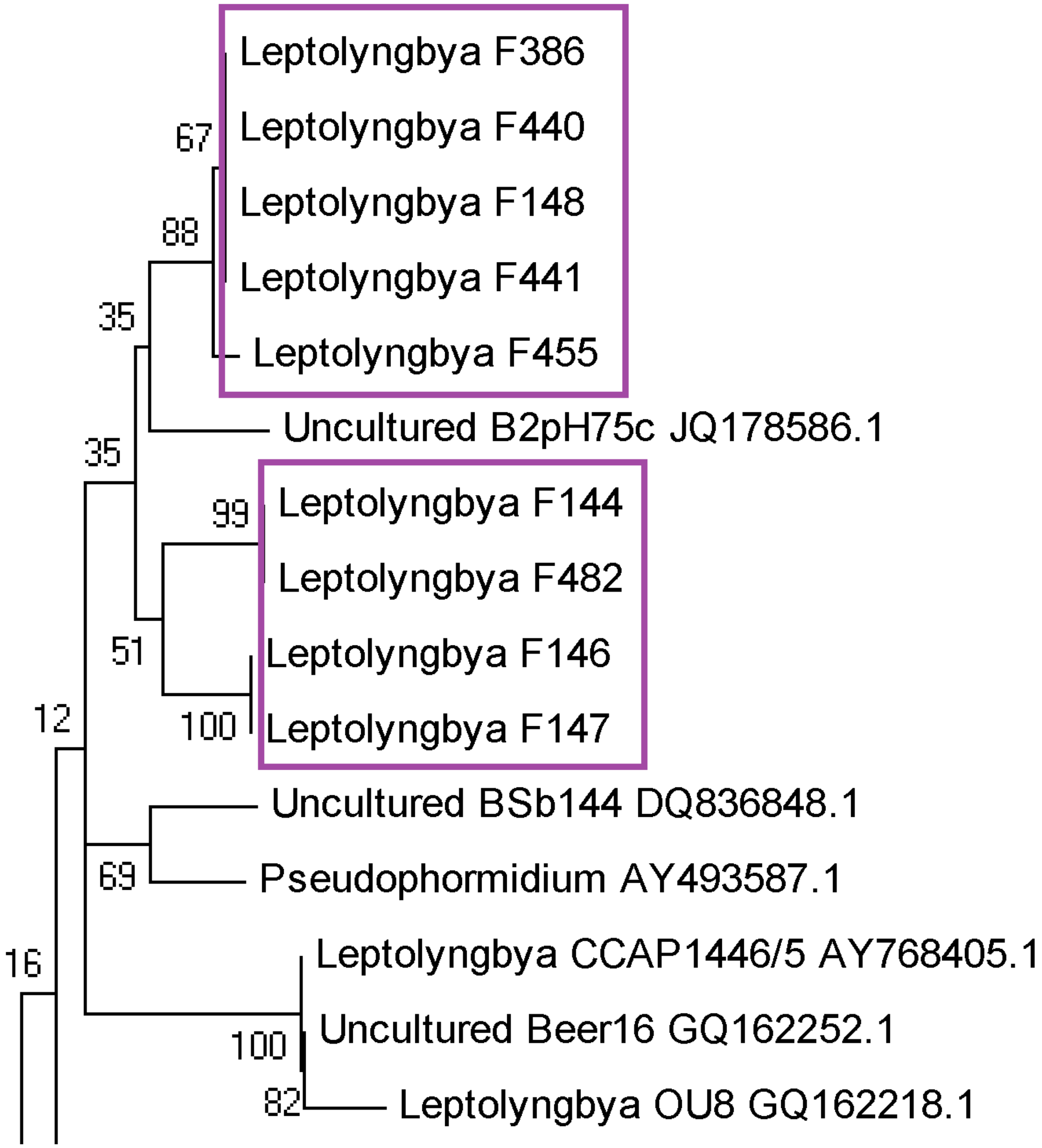

Figure 2. Neighbor-joining phylogenetic tree with 500 bootstrap resamplings for the temperate (WA, CA, and MA) *Leptolyngbya/Plectonema* algal cultures.

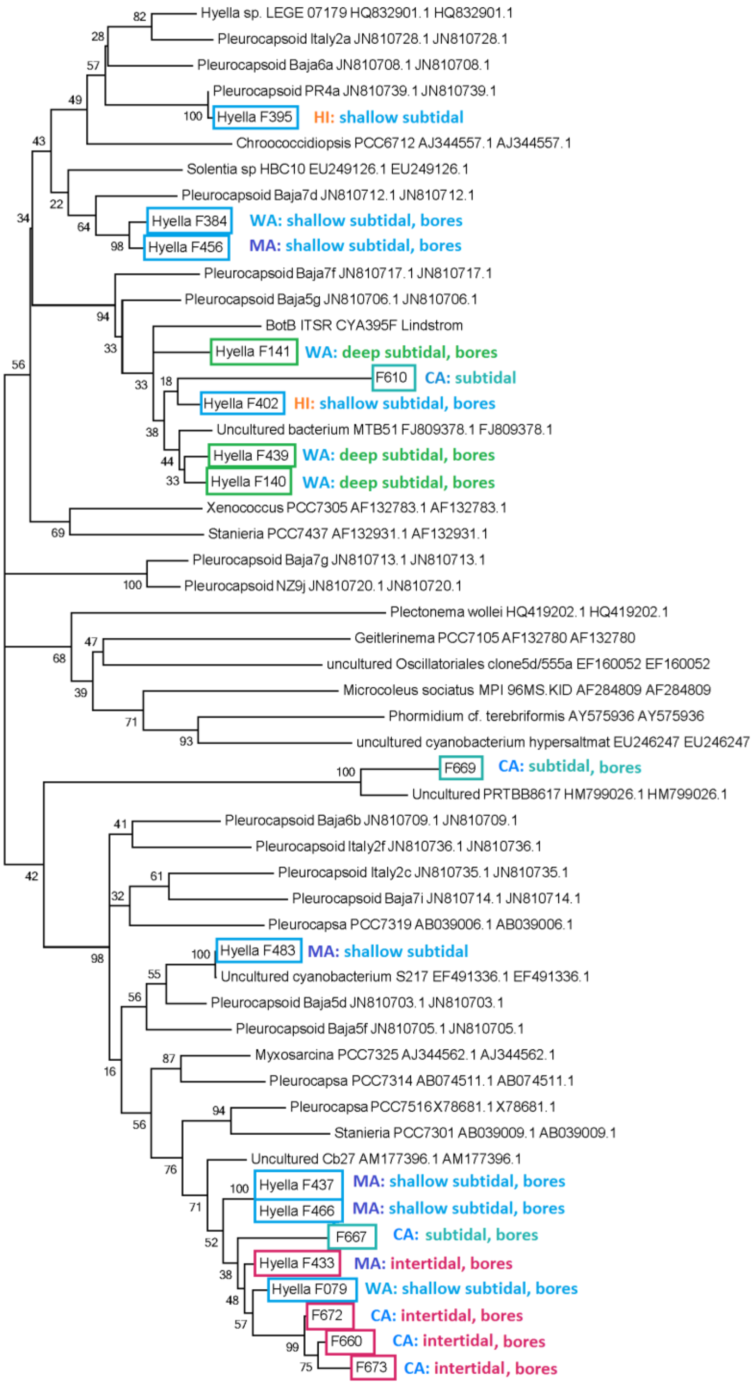

Figure 3. Neighbor-joining phylogenetic tree with 500 bootstrap resamplings for the temperate (WA, CA, and MA) *Hyella* strains.

Table 1. Qualitative abundance and mean bore trace diameter of SEM photographs from Argyle Lagoon.

	Fascichnus dactylus	*Scolecia filosa*	*Rhopalia clavigera*	*Rhopalia catenata*	*Cavernula pediculata*
Deep Subtidal	**	***		*	
Middle Subtidal	*		**		**
Shallow Subtidal		***			
Mean Diameter	7.99	3.22	8.11	6.34	37
± Standard Dev.	1.64	0.65	0.98	0.57	-

* low abundance
** medium abundance
*** high abundance