Localizing Key Bacterial Members of *Astrangia poculata*'s Microbiome and Monitoring the Changes in Taxa Diversity in Response to Holobiont Disturbance

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The facultatively symbiotic coral, *Astrangia poculata*, has a range from Buzzards Bay (RI/MA) to the Gulf of Mexico. In the northernmost population, *A. poculata* experiences seasonal temperature fluctuations from 2°C to 30°C, on average. Recent studies suggest that the *A. poculata* microbiome fluctuates according to season, but is still remarkably stable and predictable, compared to tropical corals. The highest levels of inter-colony variability in microbiome composition (beta diversity) occur in the winter months, and this is currently thought to be due to loss of regulation by the holobiont, which enters into quiescence in cold temperatures. In the spring months, as water temperatures warm, the microbiome recovers to a very predictable state that is dominated by a low number of prokaryotic taxa, including *Nitrosopumilus* sp., *Endozoicomonas* sp., *Amoebophilus* sp., and Roseobacterales. These taxa are also present in nearly all specimens that have been characterized for microbiome composition and are therefore candidate members of the *A. poculata* "core" microbiome. Based on our recent work, we hypothesize that the microbiome re-structuring in the spring is in part driven by antibacterial production by resident microbes in the *A. poculata* surface mucus layer (SML). The aim of the work proposed here was to design sequence-specific fluorescence *in situ* hybridization (FISH) probes to localize prokaryotic taxa of interest in wild symbiotic and aposymbiotic *A. poculata*, with a focus on previously identified candidate members of the *A. poculata* core microbiome and recently identified SML bacteria that inhibit growth of seawater microbes. Additionally, once localization protocols have been developed, they will be used to observe qualitative (and potentially quantitative) changes in taxa of interest in response to holobiont disturbance – for example, thermal stress, starvation, or changes in nutrient levels. FISH localization of different members of the *A. poculata* microbiome will provide new insight into microbe-microbe interactions in *A. poculata*, and their potential involvement in the response of *A. poculata* to disturbance.

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