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### Potential *Coryphella Trilineata* Cryptic Species at FHL

This observational study utilizes different forms of morphological data in an attempt to determine the presence of potential cryptic species that have been previously classified as *Coryphella trilineata*. 11 specimens were collected off the docks at Friday Harbor Labs and used in this project. They were labelled 1-11F, the F was for FHL docks as it hadn't become apparent yet that this location would be the only feasible one for collection. Each specimen was kept in a Falcon tube within a larger bin to keep them separate within the flow table. The caps of the tubes were screwed on, but each had a hole in the center and had a fine plankton net secured underneath to ensure air exchange. Their water was changed daily to prevent temperatures from rising too high. External morphological traits such as rhinophore annulation number and style, along with rhinophore and cerata coloration, were examined using a dissection scope. Each specimen had its radula dissected out using a needle and fine-tuned forceps. Only 6 radulas were able to be successfully placed onto stubs. This was due to the fact that the radulas are clear and minuscule, though not difficult to locate within the organism. The best course of action ended up being to use a Pasteur pipette to move the radulas from surface to surface. Once successfully extracted, radulas were placed into 95% ethanol while they awaited the SEM. Radulas were photographed using the SEM at varying quality due to issues with the axis alignment. Only 5 photos were taken as the 6th radula was too damaged to be useful. Furthermore, only 2 pictures were useful for identifying known anatomical features of the *C. trilineata* radula.

Additionally, 7 tissue samples from individual specimens were sent to be barcoded, but the results will take a while to be returned.

Given the fact that there were only 2 useful SEM photos radula data was not able to be properly compared and contrasted with external morphological data. As such, distinct external features between specimens were focused on and compared to the original observations from Charles O'Donoghue in 1921. Traits examined included cerata coloration, rhinophore coloration, annulation number, and annulation asymmetry (differing number of annulations of right vs left rhinophore) and, annulate rhinophore styles. All species possessed 3-4 ill-defined cerata groups behind their distinct 1st grouping, 3 iridescent white lines, conical cerata, orange cnidosacs at the

tips of cerata, body sizes within 5-35 mm, and similar general body plans. While the yellow coloration on *C. trilineata* rhinophores is described to be present at the most distal end of the clavus, this was not observed in any specimen. This reported yellow coloration on the rhinophores varied in placement on the clavus as well as in hue strength between specimens. The coloration was either not present or had differing placements on the rhinophores. Cerata coloration varied from bright red, brick red, dark orange, and bright orange. In general, coloration was not the most reliable trait, as it could shift depending on the amount and angle of light. The number of annulations among specimens fell between 4-16. The 3 styles of annulate rhinophore observed were: shallow/normal annulations (1F, 2F, 7F, 8F), pronounced annulations (3F, 5F, 6F, 10F), and overlapping annulations (9F). 11F had damaged rhinophores and so was not included in the comparison. Specimens 1F and 6F were the only individuals with asymmetrical rhinophores.

Overall, there was a presence of variability between the specimens' morphological traits, most notably among rhinophore morphology, but there were no definitive associations between these traits. Variations could be due to ontogeny, natural variation, other processes, or the existence of cryptic species. Given this data, the presence of cryptic species for *C. trilineata* can be neither confirmed nor denied, and further research is most definitely needed. Ideally, a genetic analysis of a larger sample size would aid in better determining whether these *C. trilineata* cryptic species exist.