Investigating morphological variation in Siphonophores

Background & Proposal: Historically, Siphonophores have been mistaken for jellyfish due to their transparent bodies, long tentacles, and stinging nematocysts [1], [2]. Like many other cnidarians (e.g., corals), Siphonophores are colonial animals and are made up of multiple animal bodies, called **zooids**, which arise from the same embryo and function together as one organism. Within the Siphonophore, zooid types are arranged in a specific pattern, which is repeated across the organism and determined at the growth zones of the Siphonophore [4]. Siphonophores have a high degree of functional specialization and precise organization within the colony, which sets them apart from most other animal species [3]. Though Siphonophores are a diverse group, we lack an understanding of how the organizational pattern of zooid type differs across species, and to what degree this morphological variation of patterns is conserved. Understanding the conservation of pattern type, will inform us of the functional specialization structures that are indicative of their survival. To answer these questions, I will use geometric morphometric methods to compare differences among Siphonophore species. This approach builds on **recent work done in the Casey Dunn lab at Yale**, draws directly from my **experience in Dr. Dean Adams's lab,** and is motivated by my own interest in **complex trait evolution**. Last year, the Dunn lab published a transcriptome-based Siphonophore phylogeny and used it to reconstruct the evolutionary history of changes in Siphonophore sexual systems, life history traits, habitats, and zooid types. In their comparisons of zooid type, Munro et al. [1] used only the binary characterization of presence/absence of each zooid type, making this study void of any zooid organizational pattern classification. Previous studies have also suggested that organizational patterns of zooid type are species-specific [5]. <u>These organizational patterns have never been examined from a phylogenetic perspective</u>. I am interested in extending the work done in Dr. Dunn's lab by <u>quantifying morphological variation of zooid types to determine their evolutionary history and organizational pattern within the Siphonophore colony.</u> Understanding the evolution of zooid types is key to unraveling the mechanisms behind coloniality and functional specialization. Broadly, this study will improve our understanding of complex traits in non-model organisms from which we lack critical information about their basic biology. **The aim of my study is to determine the evolution of organizational patterns and variation of zooid specialization in Siphonophores by applying novel methods to quantify three-dimensional data.** To quantify the morphological variation of zooid type beyond presence/absence descriptions, I will use micro-computerized tomography (CT) scans to characterize organizational patterns. This project will analyze traits of Siphonophores that is <u>currently not understood within the scientific community</u>.

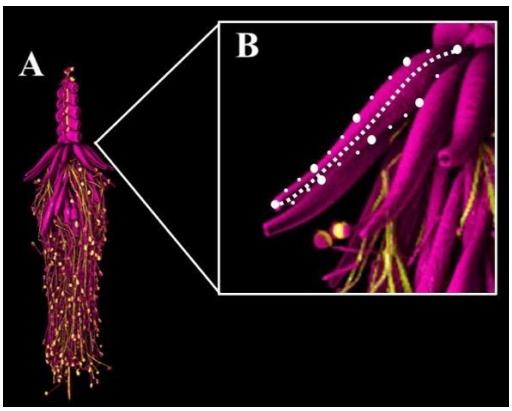

*Figure SEQ Figure * ARABIC 1 (A) Reconstructed glass Siphonophore from micro CT scan. (B) Proposed landmarks (white dots) to quantify zooid shape. Adapted from www.sciencephoto.com.*

Methods: I will collect at least three specimens for each of the 33 species analyzed in the phylogeny produced by Munro et al [1] to quantify zooid morphology. Specimens will be collected via blue water SCUBA diving or remotely operated vehicles from the Monterey Bay Aquarium Research Institute. Collected samples will be stored and preserved in solutions of formaldehyde, as standard procedure [6]. In the Dunn lab at Yale, I will stain samples using osmium tetroxide to enhance the visualization of body structures and then use the

micro-computerized tomography scanner available on site to scan collected specimens. Using CT scans, I will obtain images of x-rays for every species. To quantify zooid structures from these scans, I will develop a novel landmark scheme appropriate for use in Siphonophores and obtain these data using the program *Avizo*™ (Fig. 1). I will then perform multivariate computational analysis for these landmarks using the *geomorph* package [7] in R [8]. To test for correlations between zooid morphology and phylogenetic history, I will perform a phylogenetic regression for Procrustes shape variables, which will identify patterns of zooid shape variation across the Siphonophore phylogeny. I will then determine the rate of evolution for zooid shape and organizational pattern by performing morphological disparity tests. These results will indicate the tempo and mode by which these morphological structures have evolved and the degree of conservation across species.

Feasibility: In the Dunn Lab, I will work with experts in evolutionary and Siphonophore biology. At Yale, having access to the largest Siphonophore collection to date would allow me to assess all preserved specimens to incorporate into my research. My past research experience in preparing and maintaining museum specimens, as well as operating and analyzing data from a CT scanner will allow me to successfully complete this project. In the Casey Dunn Lab, I will apply methods from my work with Dr. Dean Adams's lab including geometric morphometrics, biostatistics, and phylogenetics to complete this project.

Intellectual Merit: The collections from this study will illuminate our understanding of the diversity across the Siphonophore phylogeny. It will also aid in the development of new techniques to maintain and preserve non-model specimens for the *Yale Peabody Museum*. I will use morphological data to reveal how Siphonophore phenotypes have dispersed throughout their evolutionary history. Applying computational approaches to compare morphology has been an ongoing limitation for research in evolutionary biology. This study will further develop these approaches by using a novel combination of techniques such as multivariate analyses and CT scanning. Major outcomes of this study will be the identification of species-specific organizational patterns, as well as a greater understanding of phenotypic plasticity of zooid types. These results will inform biologists on the evolution of coloniality, functional specialization, and the morphological specificity of zooids. All CT scans, specimens, and codes from these analyses will be openly accessible to scientists via data sharing platforms.

Broader Impacts: Throughout my dissertation, I will participate in public outreach and the education of young scholars in science by giving a series of presentations about my experience as a scientist, research methods, and results at the *Yale Peabody Museum*. At Yale, I will continue to participate in the Society for the Advancement of Chicanos/Hispanics and Native Americans in Science and begin working with Pathways to Science programs to help low-income, first-generation, and underrepresented students pursuing science. I plan to engage students in these programs and the public by using the unique context of museums. I will work jointly with curators to help build interactive exhibits by providing field video blogs, preserved specimens, and topics that expand upon the direct implications of my work into more generally societally relevant fields such as declining biodiversity and global climate change. Importantly, these proposed exhibits not only give a diverse public face to scientists, but also use the museum to help spark scientific curiosity in the public.

References:1) Munro et al. *Molecular Phylogenetics and Evolution* 127(2018):823-833. 2) Cooke et al *Clinical Toxicology* 3(1970):589-595. 3) Mackie, G.O. *Lower Metazoa* (1963)329-337. 4) Goetz FE. *Nanomia bijuga* whole animal and growth zones from http://commons.wikimedia.org/wiki (2018). 5) Dunn, C.W., Wagner, G.P., *Dev Genes Evol* 216(2006):743-754 6) Holst et al. *Journal of Plankton Research* 38(2016):1225-1242. 7) Adams et al. *Geomorph* (2018) R package version 3.0.6 8) R Core Team *R*(2013).