Background and proposal: Complex body plans evolve through the acquisition of heterogeneous material properties. In the late 18th century, architects began to combine rigid and flexible materials in order to achieve new levels of structural complexity in response to the limited space within urban areas. An analogous process happened in animals throughout the Cambrian explosion. During this time, complex animal morphologies diversified with the appearance of both rigid and flexible materials, such as skeletons, muscles, and fluid-filled cavities. The relationship between rigid and flexible materials and the production of diverse complex morphologies in skeletonized animals, such as arthropods and vertebrates, has been extensively studied. However, little is known about this relationship in gelatinous animals. In jellyfish (Cnidaria: Medusozoa), the underlying material properties are not well understood but still thought to play a major role in the evolution of diverse morphologies. Jellyfish have a free-living medusa stage (medusoid) with a bell that is responsible for locomotion[1]. Much of the medusa bell shape appears to be constrained to a concave ellipsoid, conserved across >2,000 species of jellyfish[1]. In stark contrast to this relatively simple medusa morphology, a unique subclade, the siphonophores (Cnidaria: Hydrozoa), exhibits morphologies well beyond this norm. Siphonophores are colonial animals that use asexual reproduction to produce physiologically integrated chains of individuals, called zooids. Siphonophores swim using retained, specialized medusoid zooids (nectophores), which propel the colony. Nectophores display astonishing diversity of extremely complex morphologies[2,3] (Fig. 1). In order to achieve this diversity, siphonophores have something typical medusae do not: ridges and facets. In other systems, the development of ridges and facets is dependent on the distinct underlying material properties[4]. Like free living medusae, nectophores are composed primarily of mesoglea, an expanded extracellular matrix, which is a mesh network comprised mostly of collagen fibers located between epidermal and gastrodermal epithelia[5,6]. Anecdotal evidence of physically handling siphonophores indicates that the mesoglea has a wide variety of elasticity and stiffness both within a nectophore and across species depending on the presence of ridges and facets. It is unknown to what extent the ridges and facets of nectophores depend on the material properties of the mesoglea, such as the density, elastic modulus, and viscous modulus. Typical medusae, without ridges and facets, have homogenous mesogleal material properties. **I hypothesize that complex nectophore morphologies with ridges and facets require heterogeneous mesogleal deposition within the nectophore.** If supported, the nectophores with complex ridges and facets will have both regions with elastic properties and regions with viscous material properties. In contrast, the nectophores that lack ridges and facets will have homogenous mesoglea with material properties comparable to typical medusae. If the core hypothesis is not supported, it would mean that material properties and nectophore morphology can vary independently. If this is the case, I propose two alternative hypotheses: (1) heterogenous mesoglea evolved first facilitating the evolution of ridges and facets, and (2) ridges and facets evolved first, and were secondarily reinforced by heterogeneous mesoglea. To test these hypotheses, I will characterize nectophore morphology and material properties to test for phylogenetic correlations. I will also use the phylogeny to model trait evolution of the

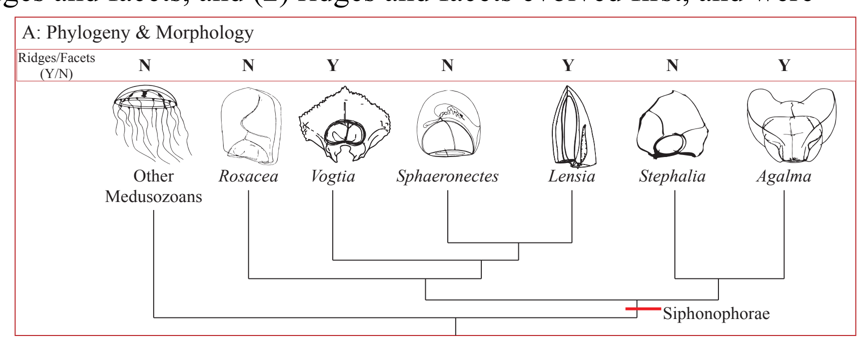

Figure 1: Phylogeny and medusa morphology. Adapted from Totton (1932).

morphology and material properties. This study will help understand not only how diversity in morphology arises, but how it evolves. Are mesogleal ultrastructure and correlated material properties phenotypically integrated with nectophore morphology? Answering this question recognizes the role material properties have in achieving morphological diversity in gelatinous animals. This work will contribute to the growing interest in the scientific community that realize diverse material properties and their critical role in the evolution of complex body plans.
***Methods**:* I will use museum specimens from the Yale Peabody Museum (YPM), which has ~180 species with ~2-3 specimens per species to describe the ridges and facets of nectophore morphology. Using literature and YPM specimens, I will delineate the presence and absence of ridges and facets across siphonophore species. Freshly collected specimens will be used for mesoglea and material property analysis. I will use scanning electron and differential interference contrast microscopy to image the mesoglea. To analyze material properties, I will use small angle x-ray scattering and rheology. These techniques characterize the structure of the mesogleal mesh at sub-micron lengthscales, density, and viscous and elastic moduli. All tools are available at Yale. Using a well resolved phylogeny[7], I will test if morphology changes in congruence with mesoglea and material properties using phylogenetic mixed models in R statistical software[8] with the *pglm* package. I will also test the order at which morphology and material properties arose via ancestral state estimation with the *ape* package.
***Feasibility**:* A risk of this project is the unpredictability of fieldwork to obtain fresh material for measurements of material properties. However, working with experts in siphonophore biology in the Dunn lab and collaborators will alleviate this risk. My previous research experience with Dr. Dean Adams in comparative morphometrics is directly applicable for questions of trait evolution. Former work at the Field Museum and current involvement in YPM has prepared me for handling museum specimens and contributing to collections for future generations. Collaborating with Dr. Alison Sweeney of Yale will contribute to the biophysical aspects of this project and her mentorship reduces radical differences between fields of physics and organismal biology.
***Intellectual Merit**:* Using and adding to YPM specimens leverage an important collection for a novel interdisciplinary project. Nectophores are a diagnostic trait of siphonophores and are used to characterize species, therefore this work will enhance what we know about siphonophore identification using gross morphology. Nectophore biology has broader implications for understanding how different zooid types contribute to colony integration. This work will also inform how extracellular matrix development has influenced metazoan evolution at early nodes in the metazoan tree, potentially with implications for body plan evolution. The emerging field of soft robotics currently use animal biomaterial properties to understand efficient fluid mechanics. A major breakthrough was a medusa-like robot. However, nectophores utilize heterogeneous soft materials to integrate a variety of functions beyond self-propulsion, allowing engineers to design robots exclusively of soft materials with extensive functional repertoires.
***Broader Impacts**:* I plan to bridge the disparate fields of physics and evolutionary biology, by sharing this work. Additionally, by being a graduate affiliate of the Yale Peabody Museum, I am able to share my research and educate about natural history to a broader audience. I will take advantage of this unique platform that encourages global curiosity about ocean exploration and overall scientific curiosity to put my work in perspective. Importantly, these opportunities excite both the scientific and public communities about current interdisciplinary research.

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