PHYLOGENETIC AND GEOGRAPHIC RELATIONSHIPS OF CHEILOSTOME BRYOZOANS IN THE EASTERN PACIFIC

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ABSTRACT

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The phylum Bryozoa is an incredibly diverse group of marine invertebrates with a widespread global distribution that is well suited for evolutionary studies but whose phylogenetic relationships are still poorly understood. Although recent studies on bryozoan taxonomies and phylogenies have increased, there is still a lack of assessment of species found at shallow water (<1 m) to intertidal depths. In this study, I aimed to expand the taxonomic sampling and assessment of the phylogenetic diversity of cheilostome bryozoans along the California coastline by utilizing mitochondrial DNA as well as inferring potential correlations between species presence and dispersal range both within and between rocky outer coast and sheltered harbor habitats. Illumina high-throughput sequencing was used to produce mitogenomes for cheilostome bryozoan samples collected off rocks from two rocky intertidal sites and off settlement panels from two harbor sites. Phylogenetic analyses generated evolutionary hypotheses of species relationships alongside geographic mapping of their distribution. This study identified 15 distinct species that represent 10 different families to form the first comprehensive phylogeny for multiple bryozoan families in California across a total range of approximately 973 km of coastline. Three genetically distinct species were found at

multiple sites that are separated by a combination of rocky shores and sandy beaches, which indicates that the dispersal range of these species are not limited by geographic barriers along the coast of California. These results provide a future opportunity for further integration of this data with the phylogenies generated in this study to examine more robust evolutionary hypotheses for the phylogenetic and geographic relationships of Californian bryozoan species.

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INTRODUCTION

Bryozoans form a taxonomically rich invertebrate phylum whose geographic distribution is widespread but whose evolution and phylogenetic relationships between and within different clades is still poorly understood. To further the understanding of bryozoan evolution, this thesis aims to develop and assess phylogenetic hypotheses among marine bryozoans along the California coastline. In the following introduction, I discuss a general overview of the phylum Bryozoa, the methods that have been used herein to generate evolutionary trees, the role of biogeography on bryozoan distribution, and the role of phylogenomics in developing evolutionary hypotheses.

Overview of Bryozoan Evolutionary History and Diversity

The phylum Bryozoa is a very large and diverse group of benthic organisms commonly referred to as moss animals. The phylum is comprised of small aquatic invertebrates and has over 6000 described extant species that are globally distributed (Bock & Gordon, 2013). They are characterized by their use of a lophophore, a ciliated organ used for both food collection and gas exchange (Taylor & Waeschenbach, 2015), which places them within the superphylum Lophotrochozoa (Halanych, 1995). They are colonial organisms, and some species possess polymorphic zooids (heterozooids). These modules are morphologically distinct from the feeding individuals (autozooids) which have lophophores, in part because heterozooids often lack feeding organs. Some polymorphic zooids possess beak-like structures (avicularia) which can ward off potential

predators, and hence it is common for polymorphic modules to have functions other than feeding (Winston, 1984). Bryozoan colonies grow by budding new modules (zooids) asexually to form a larger colony, while sexual reproduction involves the release of swimming larvae into the water column following syngamy of sperm and egg to form new, genetically distinct colonies (Taylor & Waeschenbach, 2015).

Bryozoans have many roles in the community structure of benthic ecosystems. They can act as bioconstructors by creating habitat for various invertebrate taxa including crustaceans, flatworms, juvenile bivalves, and urochordates (Cocito, 2004; Waeschenbach et al., 2012b; Wilson, 2011) and are preyed upon by predators, such as certain species of nudibranchs, pycnogonids, polychaetes, and nematodes (Lidgard, 2008). The calcified skeletons secreted by many species also contribute to carbonate sediment levels in shallow marine environments (Bone & James, 1993). Despite their various roles in benthic communities, bryozoans have generally been understudied in terms of their evolution and phylogenetic relationships and are one of the most neglected marine invertebrate phyla.

The phylum Bryozoa is comprised of three different classes: (1) Phylactolaemata, (2) Stenolaemata, and (3) Gymnolaemata (McKinney & Jackson, 1991). However, the interrelationships between these taxonomic groups have been highly debated. The class Phylactolaemata, sister group to Gymnolaemata and Stenolaemata, is only found in freshwater environments and species within this class are known for their trimeric body plan (Taylor & Waeschenbach, 2015). The class Stenolaemata, with a single extant order (Cyclostomata), is found only in marine environments and contains calcified skeletal

features, including tubular-shaped zooids. However, due to the lack of large numbers of extant species of stenolaemates, as well as the lack of phylogenetically informative morphological characters in this group, there has been great confusion concerning the phylogenetic placement of the class Stenolaemata within the phylum Bryozoa- however it is generally still identified as a monophyletic clade (Waeschenbach et al., 2012b). The class Gymnolaemata is mostly marine and contains two orders: (1) Ctenostomata, which includes bryozoans with fleshy, uncalcified zooids, and (2) Cheilostomata, which contains species with calcified skeletons. Ctenostomes, which are found in brackish and marine waters, were previously assumed to be paraphyletic to the cheilostomes but have recently been hypothesized to be ancestral to the cheilostomes (Orr et al., 2022; Waeschenbach et al., 2012b).

The order Cheilostomata includes the largest number of living bryozoan species, containing approximately 80% of extant bryozoan species (Taylor & Waeschenbach, 2015). With the first cheilostome fossil appearing in the Late Jurassic (Pohowsky, 1973), evidence suggests that cheilostomes originally had noncalcified frontal walls that were membranous and evolved the development of basally jointed spines around the frontal membrane later on (Dick et al., 2009; McKinney & Jackson, 1991). Cheilostomes have been historically divided into the suborders Anasca and Ascophora based on their frontal membrane morphology, however molecular phylogenies have found that frontal membrane morphology is not suitable for defining a phylogenetic division within the order Cheilostomata, and thus refer to anascans and ascophorans as evolutionary grades (e.g. anascan-grade or ascophoran grade) rather than clades (Dick et al., 2009; Knight et

al., 2011; Orr et al., 2021). Anascans have noncalcified frontal membranes, which leaves the frontal surface of the zooid exposed to predators in contrast to ascophorans, which possess a calcified frontal shield (Taylor & Waeschenbach, 2015) that blocks many predators from tearing out the feeding polyps.

Based on research using the mitochondrial 16S rDNA gene by Hao et al. (2005), cheilostomes were estimated to have diverged from ctenostomes approximately 245-282 million years ago (MYA) during the Permian to the Early Triassic. This evolutionary hypothesis of cheilostomes having a Paleozoic origin has since been confirmed by recent research utilizing multi-gene sampling and fossil calibrations, indicating that there was a large evolutionary period between their initial divergence from the ctenostomes and their first fossil records during the Late Jurassic (Orr et al., 2022). Rates of morphological and molecular evolution often vary between and within different lineages, but certain genes that have experienced rapid evolution can be useful in these assessments of phylogenetic divergence between different genera and in turn calculations of their evolutionary origins (Fuchs et al., 2009; Schwaninger, 2008).

When considering the evolutionary history of bryozoans, most of the available research findings are based on their rich fossil record which can be traced back to the Early Ordovician (Ma et al., 2015). Morphological characters that are often used to identify different species include the position and size of avicularia, spines, spikes, frontal shield morphology, ovicell morphology, aperture shape, and the size and shape of other polymorphic zooids (Liow et al., 2019). However, the small size of their skeletal features makes it difficult to differentiate between species with the naked eye or even at

times with stereo microscopes. Thus, scanning electron microscopy (SEM) is often utilized in the identification of different species (Mitra et al., 2013). The SEM can magnify a specimen up to 2 million times its actual size with a resolution of up to 0.4 nm, making it useful for studying the fine details of features that are smaller than can be easily observed under a light or dissecting microscope (Bozzola & Russell, 1999). Since bryozoans possess a variety of morphological forms and their distinguishing features are microscopic, it is possible that many genera contain cryptic species, even when viewed with higher magnification under SEM (Knowlton, 1993). Cryptic speciation occurs when organisms that are morphologically similar are classified as belonging to the same species but are instead genetically separate species (Trivedi et al., 2016).

Molecular Data and Phylogenomics

Although morphological character data is well supported by the fossil record, studies have shown that morphology-based phylogenies should be relied upon with caution and that phylogenies should also be based upon other types of data such as molecular data to formulate more robust evolutionary hypotheses for the relationships between bryozoan species (Orr et al., 2019b). Molecular genetics often use DNA barcoding to sequence a specific region of the genome in order to genetically discriminate between different species (Trivedi et al., 2016). This method can potentially identify all of the genera, if not species, present in a particular community as well as cryptic species which are sometimes impossible to discern even under an electron microscope (Smith et al., 2011).

Mitochondrial genes are often used in species identification because they are passed down to offspring through maternal inheritance and often include protein-coding genes. These genes have a high number of copies within the mitochondrion, often lack introns (although recent research has found the presence of introns in several cheilostome families), have high substitution rates (in comparison to the lower substitution rates found in nuclear genes), and have no recombination, which all contribute to the reduction of the number of sources of change within the nucleotide sequence while still providing identifiable sequence diversity (Jenkins et al., 2021; Raupach et al., 2015). The cytochrome oxidase I (COI) mitochondrial gene is commonly used as a molecular marker in metazoan evolutionary studies because it evolves in a relatively short time frame (generation time of a few years in invertebrate species) and is often easily recovered in DNA sequencing which is useful for species identification (Ge et al., 2021; Thomas et al., 2010). However, due to the high interspecies sequence variability of bryozoans, it should be recognized that adhering to a universal threshold for gene sequence identity (including COI) may not be suitable for determining all species and/or genera of bryozoans (Orr et al., 2019a). Since genetic similarity may be over- or underestimated, the same sequence identity thresholds should not be assumed to be applicable for every taxonomic group and should therefore be determined from robustly sampled datasets (Lee et al., 2011; Orr et al., 2019a).

Similar to mitochondrial genes, nuclear genes can also be used as DNA barcodes. Nuclear genes, which also include some non-protein-coding genes, are often slower to evolve than mitochondrial genes, such as in the 18S ribosomal RNA (rRNA) gene (Wilke

et al., 2009). While phylogenetic trees can be built using a single gene (e.g. COI) from DNA barcoding, species level molecular phylogenies should include multiple genes because the molecular rate of evolution will vary for each gene within a species and the use of larger molecular datasets can provide a more robust phylogenetic inference of the evolutionary relationships between species (Duchêne et al., 2011). The use of complete or nearly complete mitogenomes (a.k.a. mitochondrial genomes) alone can produce phylogenies with much greater resolution and precision over ones developed from only a few targeted genes (Duchêne et al., 2011; Trevisan et al., 2019). Conserved nuclear genes are also used to resolve some of the deeper nodes of phylogenetic trees, making them useful in evolutionary genetic studies of ancient groups (Luo et al., 2019). Many studies that use multi-gene molecular phylogenies are able to not only expand the available dataset of sequences available but also better resolve the interrelationships for which taxonomic classifications should be based (Waeschenbach et al., 2012b). This is a vital part of the study of phylogenomics, which seeks to use multispecies phylogenetic comparisons to infer phylogenetic relationships between taxa and gain insights on their molecular evolution from genomic-scale data, rather than through the use of only a few genes (Young & Gillung, 2019).

Traditional methods involving the use of the polymerase chain reaction (PCR) to amplify the DNA barcode region and subsequent Sanger sequencing in order to generate enough sequences to build a phylogeny can be exhausting and time-consuming. Since DNA barcoding requires the targeting of a specific gene fragment, the amplification process leads to the risk of sequence heterogeneity loss as a result of modifying the

number of PCR cycles, over-amplification of shorter fragments, GC:AT content, and DNA to Taq polymerase bias (Robin et al., 2016). The immense DNA sequence variability between bryozoans requires multiple primers to be designed which can lead to issues with targeting and amplifying a barcode region, especially if the DNA is already fragmented prior to targeting. The generally small colony size of bryozoans as well as their close proximity to other biota (including other bryozoans since different colonies often grow on top of each other) also provides challenges to obtain good molecular sequences due to the ease of contamination with non-bryozoan DNA or the DNA of the wrong bryozoan species, especially when using PCR based techniques (Orr et al., 2019b). Advances in genomic technology provide a way to overcome some of the effects of stochastic error in phylogenetic analysis that is associated with the limitations of the low number of loci analyzed in Sanger sequencing (Young & Gillung, 2019). In particular, "genome skimming" provides a method to rapidly perform shallow sequencing of the entire genome as well as quantify the number of times each nucleotide base is sequenced (Straub et al., 2012).

Next generation sequencing (NGS), also known as high throughput sequencing (HTS), is a direct sequencing technique used for genome skimming that uses highly parallelized processes to sequence thousands to millions of molecules simultaneously (in comparison to Sanger sequencing, which utilizes chain-termination sequencing) (Al-Haggar et al., 2013). By "skimming" the genome using HTS, scientists are able to quickly perform shallow sequencing of entire genomes of multiple species at once (Denver et al., 2016; Trevisan et al., 2019). This reduces the amount of time spent

troubleshooting samples with low DNA yield or amplification processes with low specificity that may come with PCR methods used on one or a few individual genes (e.g., COI barcoding). Since genome skimming results in a high relative abundance of total genomic DNA due to the capture of high copy genes (e.g. mitochondrial and housekeeping genes), low coverage genome sequencing can also be used to characterize conserved nuclear loci (Straub et al., 2012).

Biogeography of the Northeast Pacific

Studies have found that the historic and modern geographic ranges, as well as population structure of different marine species, are often influenced by the physical geography of their habitat (Kelly & Palumbi, 2010). While these geographical distributions may be the result of the presence of physical barriers (e.g. cliffs), distribution patterns are often formed across other types of geographic gradients including latitude and depth (Lomolino et al., 2010).

The study of marine biogeography first began to emerge in the mid-19th century when the species ranges of mollusks, corals, and crustaceans were used to define geographical boundaries for marine provinces (Dana, 1853; Woodward, 1851). Studies of marine taxa in temperate regions of the Pacific Ocean have found distribution patterns in a variety of species whose evolutionary divergences are influenced by a range of dispersal capabilities, temperature tolerances, and climate changes (Bowen et al., 2016; Grant et al., 2012). Phylogeographic analysis can be used to study distribution patterns in different species by using genetic data alongside time scales and biogeography to map

trends in genealogical lineages (Avise, 2000). Genetic markers, especially mitochondrial markers, are often used alongside biogeographic data to infer phylogeographic patterns in a given population (Avise et al., 1987; Wright, 1943). Since the cryptic speciation of some bryozoan taxa can make identifying distinct species difficult, molecular data is often necessary to identify which species are present in a geographic population.

The geography of the northeastern Pacific coastline provides numerous opportunities to study the marine diversity of various intertidal communities, especially in the United States where bryozoans have been vastly understudied in comparison to other marine taxa (Kelly & Palumbi, 2010; Sanford et al., 2019). The Pacific coast of the continental United States is part of the Oregonian and Californian Provinces, two large biogeographic regions which can be divided into several sub-provinces as defined by Hall and Valentine's studies on molluscan species ranges (Hall, 1964; Valentine, 1966). The majority of the Oregonian Province encompasses two sub-provinces known as the Mendocinian (from Cape Flattery, Washington to the northern end of Monterey Bay, California) and the Montereyan (from Monterey Bay to Point Conception, California) sub-province regions (Blanchette et al., 2008; Muhs et al., 2014). The Californian Province (which includes the Southern California Bight) then stretches from the southern edge of the Oregonian Province at Point Conception south to Punta Eugenia, Baja California Sur (Claisse et al., 2018; Muhs et al., 2014). The Californian Province can be further subdivided into the Southern Californian (Point Conception to Santa Monica Bay) and the Ensenadian (Santa Monica Bay to Punta Eugenia) sub-province regions (Blanchette et al., 2008; Valentine, 1966). The coastline along both the Oregonian and

Californian Province is characterized by various geographical features including long stretches of sandy and rocky shores, estuaries, and headlands which may influence the defining characteristics of each biogeographic province as well as local community structure (Blanchette et al., 2008; Claisse et al., 2018; Ruggiero et al., 2013).

Bryozoan Distribution and Dispersal Mechanisms

The phylogeographic relationships between different communities and subsequently the amount of gene flow among populations can be impacted by the presence of physical geographic barriers. Headlands or capes such as Cape Mendocino in northern California have been hypothesized to act as transition zones for some marine species (Connolly et al., 2001) due to the sharp differences in currents across them. Most notably, Point Conception has historically been hypothesized to act as a marine biogeographic transition zone due to its placement between the colder California Current in the north and warmer Southern California Countercurrent flowing from the south (Claisse et al., 2018). Since most bryozoans, like other sessile marine invertebrates (such as barnacles), reproduce via releasing sperm into the water column to fertilize the eggs (followed by brooding of eggs in most bryozoans), any barrier that either prevents the mixing of sperm and eggs or prevents the settlement of larvae on a substrate can influence the reproductive success of members of the population and subsequent gene flow across populations (Bishop, 1998).

Many cheilostome bryozoan larvae are lecithotrophic and generally do not disperse far from their parental source colony, so the lack of long-distance dispersal in

most species may affect the overall phylogeographic range of a particular genus or species (and can therefore influence local population structure and speciation). However, many species have been known to settle on the outside of ship hulls and/or be taken up in ballast water tanks of commercial vessels, thus allowing for larval dispersal outside of their natural region and further the promotion of gene flow to new areas (Holland, 2000). Since bryozoan larvae are often released into the water column, some species are competent invaders if those larvae settle on ship hulls or in ballast tanks and are carried to new bays or harbors (Mackie et al., 2006). While it is known that certain species (e.g. *Bugula neritina* and *Watersipora subtorquata*) can invade different bays via transport on ships, there is relatively little knowledge of the ability of bryozoan species to spread across different intertidal areas on the outer coast or between the rocky outer coast and bays (Mackie et al., 2006).

Bryozoans found within the rocky intertidal zone (an area known to have the most abundant and diverse bryozoan fauna in comparison to the subtidal zone) are likely to experience more stress and unpredictability within their environment in comparison to species found in lakes or bays (Dick et al., 2005). The rocky intertidal is the region of the shoreline that exists between the highest and lowest tide marks (Ricketts & Calvin, 1968). Species that inhabit intertidal areas often deal with mixed semi-diurnal tides (one to two unequal low and high tides a day) on the west coast of the U.S.A., which causes species found in the upper and middle intertidal to experience more exposure to air and thus desiccation stress than those found in the lower intertidal or deep in the subtidal (Valdivia et al., 2011). The subtidal zone is almost entirely submerged and exists beneath

the low tide line, therefore lessening the daily effects of exposure from the tides (Ricketts & Calvin, 1968).

Differences in the level of physical stress can impact bryozoan morphologies and thus influence certain species to be found in specific habitats. Bryozoans found in high disturbance areas (e.g. the outer coast rocky intertidal) often have encrusting rather than erect growth forms because they are less likely to break off the substrate or be consumed by predators, leading to a higher distribution of encrusting species that could potentially disperse from intertidal areas (McKinney & Jackson, 1991). Species found in low disturbance areas, including those found on the undersides of boats or floating docks (which are designed to rise and fall with the tide level and therefore remain submerged) in harbor habitats, could support the growth of species (e.g. bryozoans with erect or arborescent morphologies) that may be less likely to survive in the constantly changing conditions of the intertidal zone on the rocky outer coast (Kozloff, 1983).

Role of Phylogenomics in Bryozoan Evolutionary Hypotheses

Given the wide diversity and global abundance of bryozoans, there is still much room for further research to be performed on this phylum to better understand the evolution of cheilostomes and their respective phylogenetic relationships (Orr et al., 2021). Some studies have already begun to utilize HTS sequencing to improve the quality and length of molecular sequence data to resolve the taxonomical ranking of certain bryozoan taxa. For example, HTS sequencing was used to discover that the genera *Microporella* and *Fenestrulina* within the family Microporellidae were separate

monophyletic groups rather than confamilial as previously believed (Orr et al., 2019b). Many bryozoan taxa, including Microporellidae, have been found to have inconsistencies in their taxonomic classification and character differentiation which require a further need to expand the available molecular and morphological evidence to resolve disputes with their phylogenetic relationships (Tilbrook, 2006).

Despite the rich coastline with multiple types of marine invertebrate communities, relatively few studies have assessed the taxonomy and biodiversity of bryozoans within the northeastern Pacific and even fewer studies have examined intertidal species. The last major taxonomic studies of bryozoans along the North American Pacific coastline were performed in the late 1990's and early 2000's on primarily subtidal species (Soule et al., 2007). Therefore, it is likely that there may be several undiscovered species in our region whose phyletic inclusion in bryozoan taxonomies can be valuable to bryozoan taxonomists (as well as marine conservationists) studying these invertebrate community assemblages.

The combination of genomic, morphological, and biogeographical data can be used to not only understand an organism's evolutionary history, but also infer what may have driven the evolutionary changes seen in the organisms (Schwaninger, 2008). In addition, imbalances between the strength of diversifying selection and dispersal potential across populations can also lead to spatial differences within a genetic cline (Sotka et al., 2004). Therefore, it is hypothesized that the evolutionary relationships between local bryozoan taxa will vary between different regions due to separation by stretches of sandy and rocky shores which could restrict potential dispersal and allow

isolated populations to be free to diverge through time as well as due to the introduction of different species to other regions via long-distance dispersal from anthropogenic sources.

The main objectives of this investigation are to utilize mitochondrial DNA as well as geographic distribution data to assess the evolutionary relationships of cheilostome bryozoans found throughout the rocky intertidal and bays along the California coastline. This study is based on collections of cheilostome bryozoans, whose erect or encrusting colonies are commonly found on small rocks (which can be easily cut off and removed for sampling), from two rocky intertidal sites separated by a combination of sandy and rocky shoreline from Humboldt County, California. Bryozoan samples from subtidal sites in harbors in Morro Bay and Long Beach, California, which were previously collected by the Smithsonian Environmental Research Center (SERC), were also included in this study to expand the available diversity and geographic range of the dataset as well as to examine the phylogenetic relationships of species found on the rocky outer coastline versus those found in protected bays and harbors. The two harbor sites were added to the study due to complications caused by the COVID-19 pandemic preventing the further analysis of bryozoan samples collected from 15 additional rocky outer coast sites (between Crescent City, California and Estero Bay, California) other than the two included from Humboldt County, which led to modification of the original geographic range of the study to include only two rocky intertidal sites from Humboldt County as well as the two harbor sites in this thesis. To complete this work, I collaborated with researchers at the Natural History Museum at the University of Oslo, Norway and the

Norwegian Sequencing Centre to perform high throughput sequencing of collected samples. The phylogenetic relationships of the bryozoan samples collected were assessed using Maximum Likelihood and Bayesian inference methods. The geographic distribution of bryozoan species along the California coastline was also assessed for potential correlations between species presence at each habitat type and site and possible dispersal range. The aim of this assessment of both phylogenetic and geographic relationships of cheilostome bryozoans is to further the understanding of the speciation and spread of bryozoans throughout the eastern Pacific.

METHODS

Sample Collection Sites

I collected samples of cheilostome bryozoans at two rocky intertidal sites located on the outer coast of Humboldt County, California, U.S.A. during the period between November and December 2019 (Figure 1, Table 1, and Appendix A). Baker Beach (41.049 N, -124.126 E) is a relatively secluded cove located approximately 2 km south of Trinidad Head (Figures 2 & 3). Sampling occurred there on three separate occasions on November 10, 23, and 24 of 2019. The intertidal region at Baker Beach is a semi-protected area characterized by large sea stacks at a peninsular-like point that is surrounded by a large boulder field. Sampling was restricted to the southern, more protected side of that boulder field. The second collecting site, Palmer's Point (41.131 N, -124.164 E), is an exposed boulder field located approximately 10 km north of Baker Beach and is found within Sue-meg State Park (formerly known as Patrick's Point State Park; see Figures 2 & 4). The intertidal region there is characterized by a large gently sloping field of small to medium-sized boulders with a few giant sedentary boulders. Sampling at Palmer's Point occurred only on December 9, 2019 due to weather restrictions.

I also obtained samples of cheilostome bryozoans from protected harbors or marinas that were collected by the Smithsonian Environmental Research Center (SERC: see Figures 1 & 2 and Table 1). These samples were collected from PVC settlement

plates placed at marinas in four different localities within Morro Bay in 2013 and one locality in Long Beach in 2017. Harbor sites will henceforth be referred to by the city name (either Morro Bay or Long Beach) rather than the individual marina (locality) due to the close proximity between the four localities within Morro Bay or due to having only one marina sampled (Long Beach).

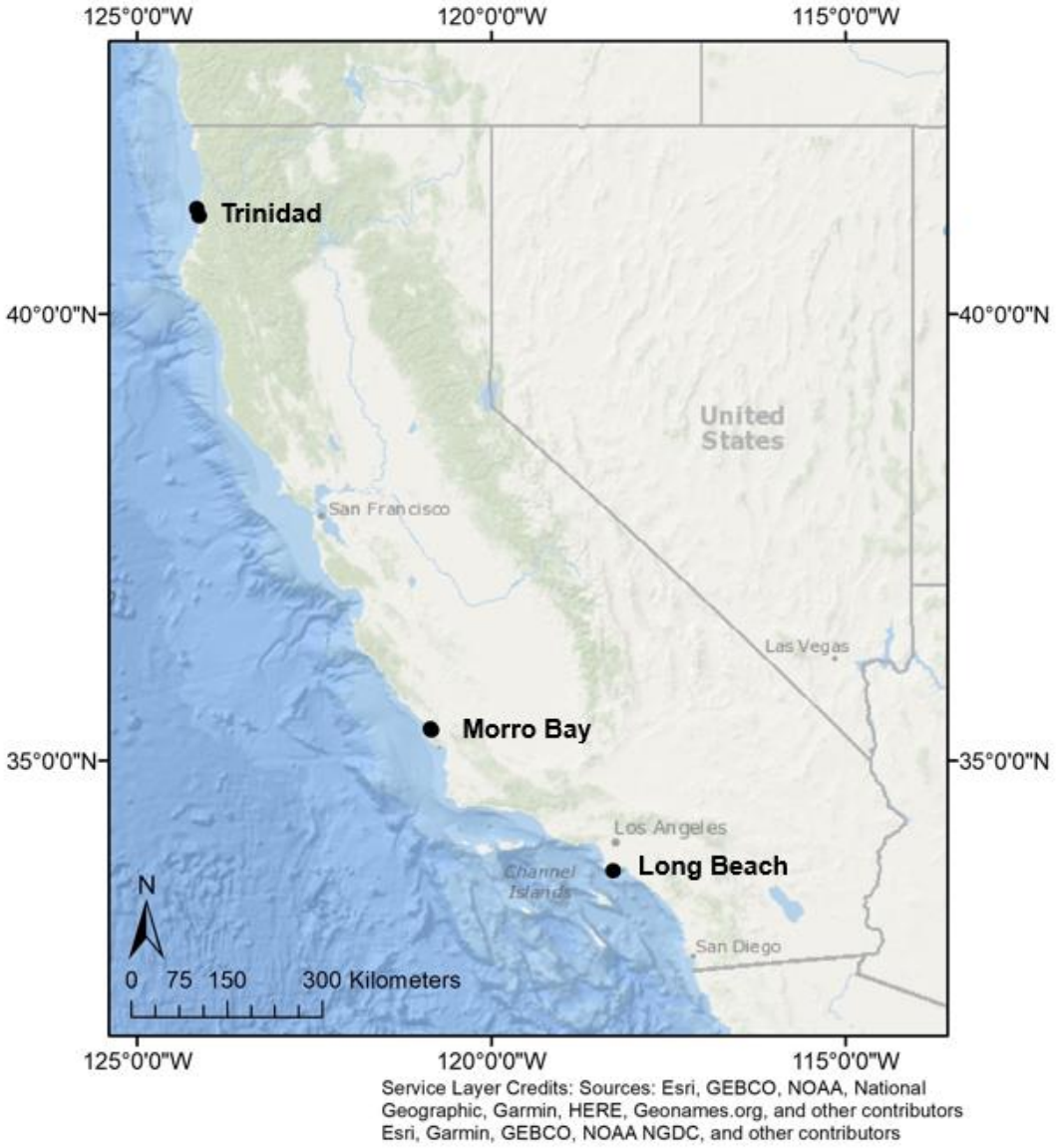

Figure 1: Map of sample collection sites along the California coastline. Two sites were sampled from the rocky outer coast near Trinidad. At the harbor sites, Morro Bay had samples collected from four localities within the site and Long Beach had one locality within the site. Sample sites are individually highlighted in Figure 2.

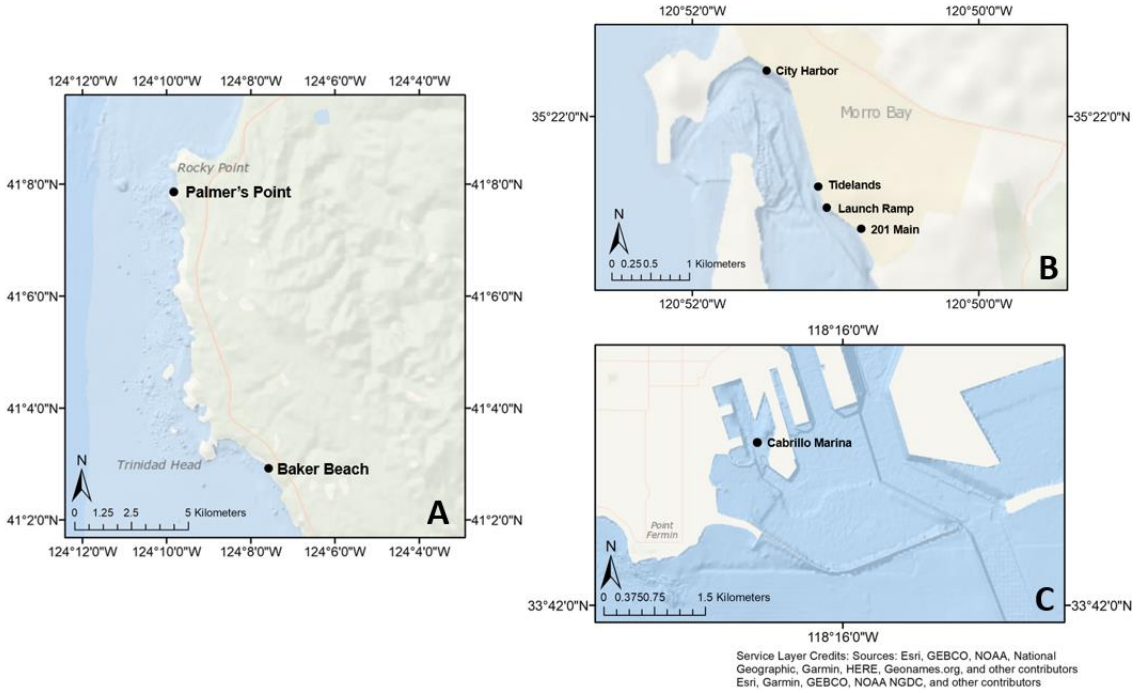

Figure 2: Map of individual sample collection sites in California based on geographic region (as defined by the nearest city). A) Trinidad (includes rocky intertidal sites Palmer's Point and Baker Beach); B) Morro Bay (includes harbor localities City Harbor, Tidelands, Launch Ramp, and 201 Main); and C) Long Beach (includes harbor locality Cabrillo Marina). Coordinates for each site correspond to those listed in Table 1.

Figure 3: Baker Beach is a small cove characterized by large sea stacks at a peninsular-like point that is surrounded by a large field of small to medium-sized boulders. The site is located approximately 2 km south of Trinidad Head.

Figure 4: Palmer's Point is a large gently sloping field of small to medium-sized boulders with a few giant sedentary boulders. The site is located approximately 10 km north of Baker Beach and is found within Sue-meg State Park.

Table 1: Location of specimen collection sites in California. The collection site or locality, city, and collection date are given as well as descriptions of latitude and longitude. Samples collected in 2019 were retrieved from boulders within the rocky intertidal. Samples collected between 2013-2017 were retrieved from settlement panels within protected harbors by the Smithsonian Environmental Research Center (SERC).

Site	City	Collection Date	Latitude	Longitude
Palmer's Point	Trinidad	Dec 2019	41.131	-124.164
Baker Beach	Trinidad	Nov 2019	41.049	-124.126
City Harbor	Morro Bay	Aug 2013	35.371	-120.858
201 Main	Morro Bay	Aug 2013	35.356	-120.847
Launch Ramp	Morro Bay	Sept 2013	35.358	-120.851
Tidelands	Morro Bay	Sept 2013	35.360	-120.852
Cabrillo Marina	Long Beach	2017	33.718	-118.278

Sampling Methods

Outer coast samples were collected at low tides from small rocks and boulders found in the low intertidal zone at each site. Intertidal zones are generally defined based on the amount of exposure to the tides, which also influences the number of organisms found in each zone (Ricketts & Calvin, 1968). The low intertidal zone is an area that is exposed whenever the maximum low tide falls below the mean sea level and is often indicated by the presence of surfgrass and kelp (Ricketts & Calvin, 1968). A higher diversity of marine organisms is found in the lower intertidal zone due to less disturbance by wave exposure and less frequent exposure to air. At each collecting site on a given date, approximately 10 different rocks from the low intertidal zone were randomly

sampled for bryozoan colonies by removing a sample of each different colony found on a given rock. Rocks ranged in size from 12-75 cm in diameter and were only counted as part of the sampling effort if bryozoan colonies were present. Upon collection, all bryozoan samples were catalogued individually in the field with a unique identification code based on location and were air dried or stored in 95% or 200 proof ethanol. Encrusting bryozoans that were attached to the undersides of rocks were chipped/cut off the rock using a portable Dremel tool and a hammer and chisel. All samples collected from Palmer's Point and Baker Beach were kept at California State Polytechnic University, Humboldt (a.k.a. Cal Poly Humboldt) and later shipped for further genetic processing at the Bryozoan Lab for Ecology, Evolution and Development (a.k.a. BLEED) at the Natural History Museum at the University of Oslo in Oslo, Norway.

Bryozoans found at shallow subtidal depths in protected harbors or marinas were collected from settlement panels suspended one meter below randomly chosen floating docks in each marina by the Smithsonian Environmental Research Center (SERC: see Table 1). Settlement plates consisted of bare, dark gray, lightly sanded PVC plates measuring 13.7 × 13.7 cm and were attached to bricks with the experimental surface facing downward, parallel to the seafloor, to mimic floating docks (Marraffini et al., 2017). Plates were suspended for approximately three months during the summer (June to September) to coincide with the period of high seasonal recruitment and provided sufficient time for mature communities to develop (Jimenez et al., 2018; Marraffini et al., 2017). All plates were collected whole in August or September of 2013 & 2017, with 10 plates taken from each marina. Sessile marine invertebrates on each panel were examined

live under a dissecting microscope in the lab and each morphospecies was identified to the lowest possible taxonomic level based on morphology. Upon collection, bryozoan samples to be used for genetic analysis were preserved in 95% ethanol at SERC and later shipped for further genetic processing at the BLEED laboratory in Oslo, Norway.

Samples were exported to Norway according to the United States Postal Service (USPS) protocols and DNA processing and sequencing was conducted at the BLEED laboratory at the University of Oslo. Each colony was subsampled for both DNA isolation and for SEM for morphological examination. Morphological vouchers from all sequenced colonies were dried, bleached in diluted household bleach to remove soft tissues, and prepped according to standard SEM protocols. All SEM images were taken at the University of Oslo so that skeletal features could be distinguished and measured for morphological taxonomic identification.

Due to differences in the exposure (intertidal versus subtidal), number of sampling periods, and sampling methods used to collect samples from rocky intertidal sites by collectors from Cal Poly Humboldt versus the samples collected at harbor sites by SERC, direct comparisons of bryozoan species richness found at intertidal verses harbor sites could not be performed. The surface area of intertidal rocks was often greater than the surface area examined on settlement panels, but there was much variation in the sizes of the rocks on which bryozoans were found. Settlement panels were also not solely sampled for bryozoans, unlike the intertidal rocks, due to differences in the research goals of the collectors.

DNA Isolation & Sequencing

Ethanol-preserved samples were dried and then rinsed in phosphate-buffered saline before isolating genomic DNA using the DNeasy Blood and Tissue kit following the manufacturer's instructions (QIAGEN, Germantown, MD, USA). Colonies were homogenized in lysis buffer, using a pestle, in the presence of proteinase K (50 µg/mL). DNA templates were sequenced directly by high-throughput sequencing (HTS) at the Norwegian Sequencing Centre (Oslo, Norway) using Illumina HiSeq4000 150 bp paired-end (PE) sequencing with a 350 bp insert size.

Sequence Assembly & Alignment

The bioinformatics pipeline utilized in this study is a modified version of the one used in studies by Orr et al. (2020; 2021). Since some of the bioinformatic software used in the original bioinformatic method (e.g. SPAdes) required considerable computational resources (>100gb RAM) a new pipeline was developed utilizing software with lower memory requirements.

Illumina HiSeq reads were quality checked using FastQC v.0.11.9 (Andrews, 2010), then quality and adapter trimmed using TrimGalore v.0.4.4 (Krueger, 2015) with a quality score cut off of 35 and a minimum length of 100. Trimmed reads were assembled with MEGAHIT v.1.2.9 (Li et al., 2015) using k-mers of 21, 33, 55, 77, 99 and 127. Orthologous genomic sequences for the mitogenome were identified using blastn in

NCBI BLAST (Altschul et al., 1990). A seed-extension based assembly was then performed with Novoplasty 4.1 (Dierckxsens et al., 2017).

Mitogenome sequences were annotated with Mitos 2 using a metazoan reference and invertebrate genetic code (Bernt et al., 2013) to identify 15 mitochondrial genes: two rRNA genes (rrnL and rrnS) and 13 protein coding genes (atp6, atp8, cox1, cox2, cox3, cob, nad1, nad2, nad3, nad4, nad4l, nad5, and nad6). Suitable orthologous sequences for each gene deposited in the NCBInr database were downloaded and aligned with MAFFT v.7 (Katoh & Standley, 2013) using the respective default parameters for rRNA and protein-coding genes. All ingroup sequences were aligned with five ctenostome sequences (*Alcyonidioides mytili*, *Amathia citrina*, *Anguinella palmata*, *Flustrellidra hispida*, and *Paludicella sp*.) obtained from NCBI as the outgroup.

Each alignment was edited manually using MESQUITE v.3.61 (Maddison & Maddison, 2018). Ambiguously aligned characters were removed from each alignment using Gblocks (Talavera & Castresana, 2007) with the least stringent parameters. Each single-gene alignment was concatenated using catfasta2phyml perl script (Nylander, 2010). A total of 31 cheilostome sequences obtained from this study were used in each alignment.

Phylogenetic Analysis

Maximum likelihood (ML) phylogenetic analyses were performed for each single gene using the ‘AUTO’ parameter in RAxML v.8.2.12 (Stamatakis, 2014) to establish the evolutionary model with the best fit. The general time reversible (GTR) was the preferred

model for the two rRNA genes (rrnL and rrnS), and the MtZoa was the preferred model for the thirteen protein-coding genes. The concatenated dataset, divided into separate partitions for each gene in which each will have a separate gamma distribution was analyzed using RAxML. The topology with the highest likelihood score of 100 heuristic searches was chosen as the best fit model. Bootstrap values were calculated from 500 pseudoreplicates. Taxa with unstable phylogenetic affinities were identified and removed (following previously outlined sampling rules) using MESQUITE v.3.61 based on evaluation of a 70% majority rule (MR) consensus tree.

Bayesian inference (BI) was performed using MrBayes v.3.2.7a incorporating the mtREV evolutionary model (Huelsenbeck & Ronquist, 2001; Ronquist et al., 2012). The mtREV model is a general reversible Markov model in which each substitution probability is represented by an adjustable parameter for a total of 189 adjustable parameters (Adachi & Hasegawa, 1996; Dimmic et al., 2000). The dataset was executed, as before, with a separate partition for each gene under which each will have a separate gamma distribution. Two independent runs, each with three heated and one cold Markov Chain Monte Carlo (MCMC) chain, were initiated from a random starting tree. The MCMC chains were run for 20,000,000 generations, with trees sampled every 1000th generation. The posterior probabilities and mean marginal likelihood values of these trees were calculated after the burn-in phase, which is determined from the marginal likelihood scores of the initially sampled trees. If the average standard deviation of split frequencies of the two runs is <0.01, then it indicates the convergence of the MCMC chains. Sequences that were removed from final analyses due to a failed step in the

bioinformatics pipeline along with details for the reason why can be found in Appendix C.

Geographic Distribution

A map of the geographic range covered in this study was produced using the ArcMAP 10.8.1 application in the ArcGIS 10.8.1 software (ESRI, 2020). Since the bryozoan community at each site is expected to be represented by several different clades whose lineages are not necessarily bound to a specific geographic region due to their long evolutionary history (as well as capability of dispersal and historically documented global distribution), it is hypothesized that there is no difference in the potential of each bryozoan family to be present at any site. However, samples of the same species that are found at nearby sites are hypothesized to be more closely related genetically to each other than ones from farther sites due to the assumption that genetic differentiation increases as isolation by geographic distance also increases (Avise et al., 1987; Wright, 1943). The bryozoan diversity at each site was summarized by the distribution of the number of families, genera, and species present (e.g. species richness) from the sequenced samples. The Ocean Biodiversity Information System (OBIS) and the Global Biodiversity Information Facility (GBIF) databases as well as literature studies were referenced to estimate and compare the historical observed range of each species identified in this study.

RESULTS

Phylogenetic Inference

This study successfully sequenced and assembled 20 cheilostome colonies using 1,213 nucleotide and 2,794 characters (for a combined total of 4,007 characters) from two rocky intertidal sites on the outer coast as well as two harbor sites along the California coastline. The phylogenetic analysis elucidated 15 lineages that correspond to 10 families and 12 genera (Table 2) in total. Of these 15 species, 10 species were genetically sequenced for the first time and are noted in Table 2. A summary table with individual gene availability for each sequence is provided in Appendix D. The phylogenetic placement of each colony, as well as its identity based on morphology, was confirmed to the species level by comparison to the results of Orr et al. (2020) and SEM vouchers for each sequenced colony (except for two colonies, which were confirmed to the genus level). An additional 19 cheilostome sequences from NCBI were included in the ingroup phylogenetic analysis of the concatenated dataset of all new California bryozoans sequenced to confirm their phylogenetic placement, and these were aligned with an outgroup of five ctenostome bryozoan species (See Appendix E for available metadata for taxa from NCBI). Taxa from NCBI were chosen based on their broad phylogenetic placement throughout the order Cheilostomata as well as the confirmation of the species identification from previous studies by Orr et al. (2020). NCBI sequences were also

chosen based on their inclusion of multiple mitochondrial genes (if not all of the same 15 utilized in this study) in the sequence.

A complete or circularized mitogenome (which includes all 15 mitochondrial genes) was produced for 13 of the 20 collected samples (Table 2). Another six samples also produced a nearly complete mitogenome with 14 genes but the bioinformatics pipeline was unable to recover the atp8 gene in these samples. Statistical support for nodes based on bootstrap values (BS) in the ML analysis and posterior probability (PP) in the Bayesian analysis are defined as full support (100 BS/1 PP), high support (>90 BS/0.99 PP), moderate support (>65 BS/0.95 PP), and low support (>50 BS/0.90 PP) (Enevoldsen, 2016). Most of the nodes in the phylogeny received either high support or full support by both the ML inference and the Bayesian inference (Figure 5).

Out of the 20 new sequences included in the phylogenetic analysis, 14 of the 20 cheilostome colonies were collected from the two rocky intertidal sites at Baker Beach and Palmer's Point in northern California and represent 11 species from 10 genera (seven samples from Palmer's Point and seven samples from Baker Beach). A complete or circularized mitogenome was produced for eight of the 14 samples. The remaining six cheilostome colonies that were successfully sequenced were collected from harbor sites in Morro Bay in central California and Long Beach in southern California. These samples represent six species from five genera (five samples from Morro Bay and one sample from Long Beach), and a complete mitogenome was produced for all of the samples except *Fenestrulina delicia*.

The ML and Bayesian phylogenies both found high to full support for the monophyly of each family present in the trees with the exception of two families (Figure 5). The family Calloporidae included *Tegella horrida* (BLEED 1894, 1909, and 1914), *Copidozoum adamantum* (BLEED 1910) and *Callopora lineata* (NCBI) which together formed a highly supported monophyletic group (91 BS/1 PP) while *Cauloramphus californiensis* (BLEED 1896) was polyphyletic to the rest of the family (97 BS/1 PP). The family Chaperiidae was also polyphyletic, with *Chaperiopsis patula* (BLEED 1906) separated by several families from *Patsyella acanthodes* (NCBI) with moderate ML and full Bayesian support (73 BS/1 PP). For genera with multiple species, the ML and Bayesian phylogenies inferred monophyletic groupings for 7 genera with full support (100 BS/1 PP): *Celleporella, Microporella*, *Parasmittina*, *Rhynchozoon*, *Fenestrulina*, *Tegella*, and *Pomocellaria*. The species *T. horrida*, *Pomocellaria californica*, and *Parasmittina collifera* each had multiple samples in these phylogenies and they were all monophyletic for each species, showing strong support for this method in reflecting species identity. Full ML and Bayesian support was given to *P. californica* and *P. collifera*, while *T. horrida* had high to full support between the multiple samples of the same species with the exception of the posterior probability value for the relationship between samples BLEED 1894 and BLEED 1914 which had a low support value of 0.94 in the Bayesian analysis.

Although not significant to the aim of the study, since these taxa were not a part of the newly sequenced California samples, it is noted that the inferred topology for the ML and Bayesian trees did not agree with the nodal placement of NCBI taxa

Parasmittina solenosmilioides and *Parasmittina aotea*. While the two species were supported to be a part of the clade *Parasmittina* within the family Smittinidae, the ML phylogeny found *P. solenosmilioides* (100 BS) to be basal to *P. aotea* which had low support (51 BS), however the reverse relationship in which *P. aotea* is basal to *P. solenosmilioides* was found to be fully supported (1 PP) in the Bayesian topology (See Appendix F for Bayesian topology only tree). Samples that had complete phylogenetic analysis performed but had low DNA quality were removed from the main phylogenetic analyses (above) and are available in Appendices B and D. This impacted the trees by removing one additional family and two additional species from the specimens sequenced by this study in the phylogenies. Maximum Likelihood and Bayesian inference trees including the sequences removed for low DNA quality are shown in Appendix F.

Table 2: Taxa generated and analyzed in this study. BLEED stands for Bryozoan Lab for Ecology, Evolution and Development, Natural History Museum, University of Oslo, Norway, and BLEED numbers are numerical tags for the specimens. BLEED numbers marked with a * indicate species that were not previously sequenced prior to this study. The location given for outer coast samples is the local site name and the location for harbor samples is the city name. The mitogenome (Mt) size, in base pairs (bp), are only shown if it is complete/circularized, with NO indicating that a complete mitogenome was not formed. Genes, represents the number of genes, a maximum of 15, recovered and used in the alignments for each taxon. Accession nr. refer to those uploaded to (new sequences) or retrieved from (outgroup sequences) NCBI. Sequences were aligned with a five ctenostome bryozoan outgroup (*Alcyonidioides mytili, Amathia citrina, Anguinella palmata, Flustrellidra hispida,* and *Paludicella sp*.).

Family	**Genus**	**Species**	**BLEED**	**Location**	**Mt size (bp)**	**Genes**	**Accession nbr**
Calloporidae	*Tegella*	*horrida*	1894*	Palmer's Point	NO	15	OK245189, OK245190, OK245191
Hippothoidae	*Celleporella*	*hyalina*	1895	Palmer's Point	NO	15	OK244797, OK244798
Calloporidae	*Cauloramphus*	*californiensis*	1896*	Palmer's Point	14,573	15	OK244778
Microporellidae	*Microporella*	*sp.*	1897*	Palmer's Point	13,563	14	OK245155
Phidoloporidae	*Rhynchozoon*	*cf. tumulosum*	1899	Palmer's Point	14,102	14	OK244996
Microporellidae	*Microporella*	*umbonata*	1900*	Palmer's Point	13,931	14	OK245161
Candidae	*Pomocellaria*	*californica*	1901	Palmer's Point	15,733	15	OK244975
Fenestrulinidae	*Fenestrulina*	*umbonata*	1903*	Baker Beach	14,780	14	OK244859

Family	Genus	Species	BLEED	Location	Mt size (bp)	Genes	Accession nbr
Chaperiidae	*Chaperiopsis*	*patula*	1906*	Baker Beach	NO	15	OK244804
Smittinidae	*Parasmittina*	*collifera*	1907*	Baker Beach	14,299	15	OK244944
Calloporidae	*Tegella*	*horrida*	1909*	Baker Beach	NO	12	OK245192, OK245193, OK245194
Calloporidae	*Copidozoum*	*adamantum*	1910	Baker Beach	NO	13	OK244819
Candidae	*Pomocellaria*	*californica*	1912	Baker Beach	15,733	15	OK244976
Calloporidae	*Tegella*	*horrida*	1914*	Baker Beach	NO	15	OK245195, OK245196, OK245197
Thalamoporellidae	*Thalamoporella*	*californica*	1211	Long Beach	13,928	14	OK245202
Pacificincolidae	*Primavelans*	*insculpta*	1214*	Morro Bay	17,268	14	OK244984
Smittinidae	*Parasmittina*	*collifera*	1216*	Morro Bay	14,305	15	OK244952
Smittinidae	*Parasmittina*	*sp.*	1219*	Morro Bay	14,302	15	OK244953
Fenestrulinidae	*Fenestrulina*	*delicia*	1244*	Morro Bay	NO	15	OK244853
Candidae	*Pomocellaria*	*californica*	1246	Morro Bay	15,733	15	OK244974

Family	Genus	Species	BLEED	Location	Mt size (bp)	Genes	Accession nbr
Alcyonidiidae	*Alcyonidioides*	*mytili*		Unavailable		5	JN681069, JN681102, AEV21493, AEV21531
Vesiculariidae	*Amathia*	*citrina*		Ferrol, San Felipe, Spain		4	KM373503, JN681121
Nolellidae	*Anguinella*	*palmata*		Parana, Ilha do Mel, Morro do Sabao, Brazil		4	JN681101, AJB84768, AEV21530
Flustrellidridae	*Flustrellidra*	*hispida*		Mumbles and Aberystwyth, Wales, UK		13	NC_008192
Paludicellidae	*Paludicella*	*sp.*		Unavailable		5	JN681070, JN681103, AEV21494, AEV21532

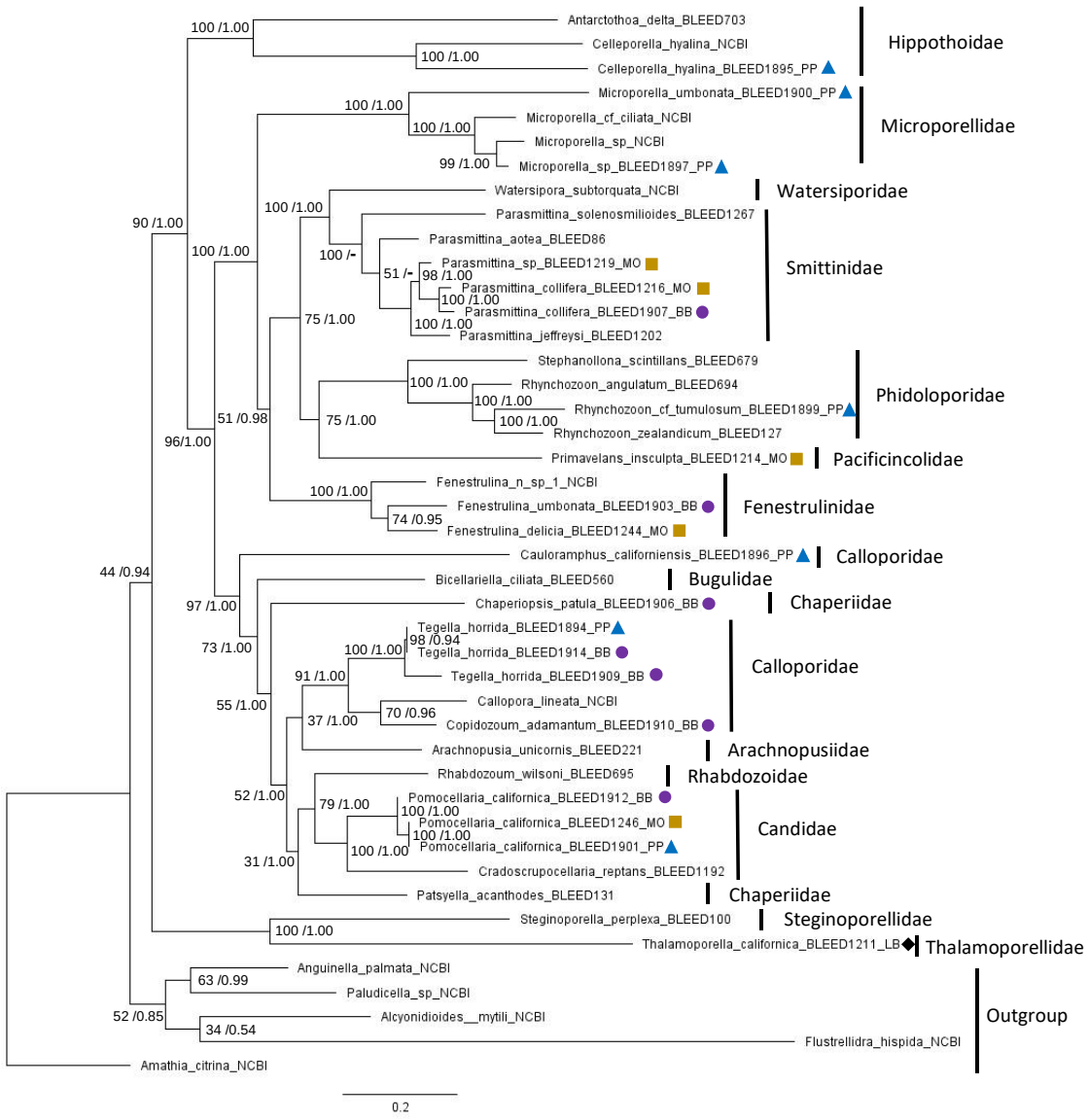

Figure 5: The phylogeny of cheilostome bryozoans collected along the California coastline between Trinidad, CA and Long Beach, CA based on 15 mitochondrial genes. Maximum likelihood topology of 39 ingroup taxa (20 taxa from this study) and 5 ctenostome outgroup taxa with 4,007 nucleotide and amino acid characters inferred using RAxML (100 heuristic searches and bootstrap values from 500 pseudoreplicates). The numbers on the internal nodes are ML bootstrap values (BS from RAxML) followed by posterior probabilities (PP from MrBayes). Black dashes indicate topological difference between the ML and Bayesian trees. Branches with samples sequenced in this study are labeled with the site abbreviation from which they were collected (PP = Palmer's Point, BB = Baker Beach, MO = Morro Bay and LB = Long Beach) and a corresponding shape. Scale bar indicates number of substitutions per site.

Geographic Distribution

The overall geographic distribution and proportion of each taxonomic family identified in this study is described in Figure 6, with a summary of the species, genera, and families found to be present at each site in Table 3. This study sampled bryozoans from four different sites (two rocky intertidal sites in northern California, one harbor site in central California, and one harbor site in southern California) over a total distance of approximately 973 km of coastline between the northernmost (Palmer's Point) and southernmost site (Long Beach).

Of the 10 different bryozoan families described, representatives of five families (Hippothoidae, Microporellidae, and Phidoloporidae at Palmer's Point, Chaperiidae at Baker Beach, and Calloporidae at both) were observed only at the two rocky outer coast sites. In comparison, only the families Pacificincolidae (in Morro Bay) and Thalamoporellidae (in Long Beach) were observed only at the harbor sites. Representatives from the families Candidae, Fenestrulinidae, and Smittinidae were found in both types of environments. This led to a total of eight families observed in the rocky intertidal areas and five families observed in the harbor areas. Samples from the families Candidae and Smittinidae each included one species that was present in both the rocky intertidal sites and the harbor sites, although the family Smittinidae had one additional species found only in Morro Bay. The two samples from the family Fenestrulinidae were found to be two different species, with *Fenestrulina umbonata* collected at Baker Beach and *F. delicia* collected in Morro Bay.

Out of the samples collected from the rocky outer coast, the combined total species richness between the two sites was found to have observed 11 distinct species while the combined total species richness from the two harbor sites was found to observe six species. The species richness was seven species at Palmer's Point, six species at Baker Beach, five species at Morro Bay, and one species at Long Beach from the samples sequenced in this study. Although direct comparisons of species richness cannot be performed due to differences in the collection methods and the number of sample days (e.g. Baker Beach was sampled three times while the other three sites were sampled once), Palmer's Point was found to be the site with the highest species richness of the sequenced samples included in the analysis despite only being sampled once.

The species *T. horrida*, *P. collifera*, and *P. californica* were each observed from multiple samples at multiple rocky intertidal and/or harbor sites. *T. horrida* was found at both rocky outer coast locations on the northern California coastline. Representatives of *P. collifera* and *P. californica* were both observed in Morro Bay and at least one northern rocky outer coast location, with *P. californica* being observed at both rocky intertidal sites. Due to the low sample sizes for each of the three species shared across all sites, statistical analysis to test for any significant changes in local genetic variation due to isolation by distance (e.g., A Mantel test) could not be performed. However, estimates of the geographic and evolutionary pairwise distances between each of the samples within a species are available in Appendix G.

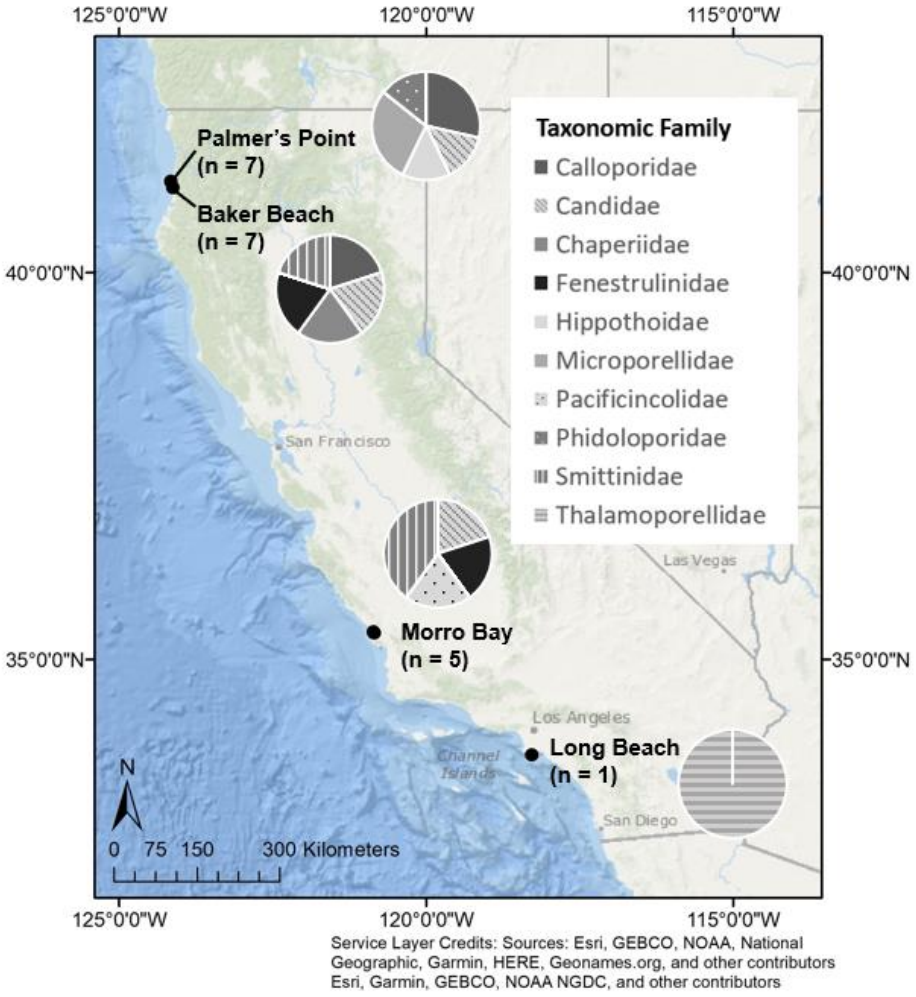

Figure 6: Map of overall taxonomic family distribution for the sequenced samples across the California coastline. Each family present at a given site is shown with its own unique color that corresponds to the legend. Pie charts represent the proportion of each family sampled at each site in the study with the total number of bryozoan samples per site indicated beneath the site name. Number of species, genera, and/or families per site can be found in Table 3.

Table 3: A summary of the total number of representative individuals sequenced for each cheilostome species (if applicable) at each site. Samples collected at Palmer's Point and Baker Beach were collected from boulders on the rocky outer coast, while those collected at Morro Bay and Long Beach were collected off settlement panels deployed within harbors. The total number of species per family is listed in the last column.

Family	**Genus**	**Species**	**Palmer's Point**	**Baker Beach**	**Morro Bay**	**Long Beach**	**Total Nbr Sp/ Family**
Calloporidae	*Cauloramphus*	*californiensis*	1	-	-	-	3
	Copidozoum	*adamantum*	-	1	-	-	
	Tegella	*horrida*	1	2	-	-	
Candidae	*Pomocellaria*	*californica*	1	1	1	-	1
Chaperiidae	*Chaperiopsis*	*patula*	-	1	-	-	1
Fenestrulinidae	*Fenestrulina*	*delicia*	-	-	1	-	2
		umbonata	-	1	-	-	
Hippothoidae	*Celleporella*	*hyalina*	1	-	-	-	1
Microporellidae	*Microporella*	*sp.*	1	-	-	-	2
		umbonata	1	-	-	-	
Pacificincolidae	*Primavelans*	*insculpta*	-	-	1	-	1
Phidoloporidae	*Rhynchozoon*	*tumulosum*	1	-	-	-	1
Smittinidae	*Parasmittina*	*sp.*	-	-	1	-	2
		collifera	-	1	1	-	
Thalamoporellidae	*Thalamoporella*	*californica*	-	-	-	1	1
		Total Number of Samples Per Site	7	7	5	1	

Morphological Species Identification and SEM

All SEM images were taken at the University of Oslo and SERC for each bryozoan sample collected in this study. Morphological identification to the lowest taxonomic level was performed for each specimen independently of the phylogenetic inference to avoid identification bias. Species identification was confirmed with the morphological descriptions of each specimen to the species level except for samples BLEED 1897 and 1219 which were only identified to the genus level. Sample BLEED 1895 was also identified to have two morphological variations for the same species. The SEM digital vouchers of all samples included in phylogenetic analysis including potential new species identified by this study as well as species that were identified to have representatives at multiple geographic locations can be found in Appendix H.

DISCUSSION

The primary goals of this study were to increase the taxonomic sampling and assessment of the phylogenetic diversity of cheilostome bryozoans along the California coastline as well as infer possible patterns of dispersal both within and between the rocky outer coast and/or sheltered harbors. The 20 cheilostome colonies that were sequenced resulted in the identification of 15 distinct species from 10 different families and 12 genera in total, indicating a wide diversity of bryozoans amongst the sites sampled and producing the first multi-family phylogenetic tree for marine bryozoans sampled from the California coastline based on phylogenomic data (Figure 5). Of the 10 families with taxa sequenced in this study, eight families were well supported in forming monophyletic families with the additional ingroup taxa from NCBI for both of the ML and Bayesian trees produced, while the remaining two families (Calloporidae and Chaperiidae) were found to be polyphyletic (Figure 5). Genera with multiple samples were also found to form monophyletic groups with full support (100 BS/1 PP) in both the ML and Bayesian phylogenies. The phylogenetic placement of the taxa to the species level (or to the genus level for BLEED 1897 and 1219) agrees with the results of Orr et al. 2020 and also supports the conclusions of Orr et al. 2019b that the genera *Fenestrulina* and *Microporella* should be placed into separate monophyletic families. A complete mitogenome (utilizing all 15 mitochondrial genes) was also produced for 13 out of the 20 new sequences, supporting the benefit that HTS sequencing can provide in reconstructing

mitogenomes for these lesser-studied taxa and thus increasing the genetic representation of bryozoan species.

The diversity of the Californian bryozoans found in this study showed that there is much variation in species distribution both within and between the rocky intertidal and/or harbor sites. Eleven distinct species were sampled between the two rocky outer coast sites, with seven species at Palmer's Point and six species at Baker Beach. Samples of *T. horrida* and *P. californica* were found at both rocky intertidal sites. A total of six distinct species were sampled at the two harbor sites, with five species in Morro Bay and one species in Long Beach. While there was some overlap between species at the various sites, the concatenated phylogenetic dataset identified five distinct species that were only found at Palmer's Point, three species only at Baker Beach, three species only found at Morro Bay, and one species solely found at Long Beach. The other three species identified in the study (*T. horrida*, *P. collifera*, and *P. californica*) were found to have overlapping geographic ranges along the California coastline, each with representatives at two to three (out of the four) different sites.

The presence of *T. horrida*, *P. collifera*, and *P. californica* at multiple sites indicates that dispersal events between these sites occurred at some point in time and allowed for gene flow between different geographical populations despite their large physical separation by headlands as well as rocky and sandy shores. Previously, these three species have been recorded in the OBIS and the GBIF databases to have observed ranges between the Channel Islands in southern California northwards to western British Columbia, Canada but until now have lacked documented presence between Monterey

Bay, California, and the central Oregon coastline except by Soule et al. (2007). Despite differences in which site each sample was collected from, a highly to fully supported group was formed for each of the three species in both the ML and Bayesian phylogenies. The phylogenetic placement of all three species within their respective clade and the identical morphology between the multiple samples for each species indicates that the samples at each site represent the same species and therefore supports the hypothesis that the species concepts for their morphology and for their phylogenetic relationships are stable for each species. Due to the low sample size for each species, a conclusive statistical test for any significant changes between genetic sequences as a result of isolation by distance could not be performed.

T. horrida was found in one sample at Palmer's Point and two samples at Baker Beach, with the relationship between the three representatives highly supported by bootstrap values of >98 and posterior probabilities of 0.94 to 1. It has been previously hypothesized that gene flow between populations that are separated by large stretches of coastline is limited as a result of isolation by distance (Wright, 1943). Given that Palmer's Point and Baker Beach are separated by a large headland as well as approximately 9.6 km of rocky and sandy shoreline, it is likely that different coves and smaller intertidal areas located between the two sites allowed for a stepping-stone pattern of gene flow to occur due to multiple dispersal events along the coastline (Fratini et al., 2016; Kimura & Weiss, 1964; Slatkin & Maddison, 1990).

P. collifera was found at both a rocky outer coast site (Baker Beach) as well as a harbor site (Morro Bay) while *P. californica* was found to be present at Palmer's Point,

Baker Beach, and Morro Bay. The relationship between the two individuals of *P. collifera* and the relationship between all three individuals of *P. californica* resulted in full phylogenetic support with bootstrap values of 100 and posterior probabilities of 1 (hence in both cases these are exactly the same species). Although *P. collifera* and *P. californica* are not historically invasive in California, these two species have the greatest geographic ranges amongst the sites observed in this study. While it is possible that a stepping-stone pattern of gene flow occurred due to short-distance dispersal events between the northern California coastline and Morro Bay, it is also possible that other mechanisms for long-distance dispersal are involved (e.g. transport on ship hulls; see Ng & Keough, 2003; Wisely, 1958, 1963).

Although most bryozoans are known to settle on hard substrates that have some stability, such as rocks or docks, encrusting species can also be found on more mobile surfaces such as marine vessels, floating algae, plastic debris, or driftwood (Avila et al., 2020; Barnes & Milner, 2005; Mackie et al., 2006). Algal holdfasts that have been disturbed by storms or tides from rocky intertidal regions have the potential to disperse bryozoans that are attached to holdfasts or algal blades farther distances via transportation along ocean currents (Avila et al., 2020). While drifting on algae or wood is likely to play a role in dispersal between shorter distances (especially between rocky outer coast regions), transportation on the hulls or in ballast waters of ships or rafting on plastic debris (which are buoyant and easily transported) could account for the larger distance (between approximately 692 to 702 km) that *P. collifera* and *P. californica* were able to

travel between the rocky intertidal sites in northern California and the harbor site in Morro Bay (Barnes & Milner, 2005; García-Gómez et al., 2021).

The overall distribution of bryozoan families was found to vary widely, no doubt in part due to the geographic location of each site and the number of individuals sampled at each site. Previous studies have recorded each family to be distributed throughout the range between the Channel Islands in southern California and western British Columbia in the OBIS and GBIF databases and in Soule et al. (2007), although there are gaps in their recorded presence between central California and central Oregon that are likely a result of low sampling in this region, especially in rocky outer coast areas. Representatives of the families Calloporidae, Chaperiidae, Hippothoidae, Microporellidae, and Phidoloporidae were only observed at the rocky outer coast sites in northern California. In comparison, the harbor sites contained two of the 10 families that were only found at the harbor sites: the family Pacificincolidae in Morro Bay and the family Thalamoporellidae in Long Beach.

Of the five families with multiple species and/or samples of a single species sequenced (Candidae, Calloporidae, Fenestrulinidae, Microporellidae, and Smittinidae), only Microporellidae (*Microporella sp*. and *M. umbonata*) and Calloporidae (*T. horrida*, *C. californiensis*, and *C. adamantum*) were found to have all collected members of the same clade present at the two rocky intertidal sites in northern California. The species *M. umbonata*, *T. horrida*, *C. californiensis*, and *C. adamantum* have been previously documented in OBIS, GBIF, and Soule et al. (2007) to be distributed in western British Columbia and from Monterey Bay to the Channel Islands, California, but their presence

in northern California to Washington, USA is still relatively undocumented. This indicates that while there may still be barriers preventing the spread of certain species of Microporellidae and Calloporidae from British Columbia towards central California, the relative dispersal and distribution of the families throughout the full range of the northeastern Pacific was not disrupted.

Representatives of the families Candidae (*P. californica*), Fenestrulinidae (*F. delicia* and *F. umbonata*), and Smittinidae (*Parasmittina sp*. and *P. collifera*) were found in both rocky intertidal and harbor areas, which suggests that these particular bryozoan families are more cosmopolitan and are not necessarily restricted to specific types of marine habitats. In particular, *F. delicia* has previously been reported in OBIS and GBIF to only be present in areas of western Europe and the northeastern Atlantic, which suggests that *F. delicia* has undergone long-distance dispersal, likely through being introduced to eastern Pacific waters anthropogenically via ships. While there are few studies comparing the difference in bryozoan diversity between rocky intertidal regions and bays, the genera *Pomocellaria*, *Fenestrulina*, and *Parasmittina* have all been historically documented to have cosmopolitan distributions with the species *P. californica, F. umbonata*, and *P. collifera* all appearing to be endemic to the Eastern Pacific (Orr et al., 2022; Soule et al., 2007; Vieira et al., 2014). Although *P. californica* and *P. collifera* were the only two species observed in both types of environments, it is likely that both short and long-distance dispersal events have allowed for multiple other species to be distributed between the California rocky outer coast regions and harbors in addition to those identified in this analysis. Species that are more ecologically tolerant of

variation in local climate as well as capable of long-distance dispersal are more likely to see a higher frequency of dispersal and subsequent gene flow and therefore a wider geographic distribution (Mackie et al., 2012; McKinney & Jackson, 1991).

Substrate availability is likely to also have a significant role in the distribution and abundance of certain species across sites. Species that were only present in marinas or harbors may be better suited than those found in rocky intertidal locations to settle on artificial substrates (e.g. boat hulls, settlement panels) (McKinney & Jackson, 1991). Some species may also be less likely to survive in intertidal locations due to more stressful conditions including greater fluctuations in wave exposure and air emersion compared to the calmer conditions in bays and harbors (Ricketts & Calvin, 1968). Since the physical geography of bays often protects the coastline from storm damage, it can be hypothesized that species with erect or more vertical arborescent morphologies are more likely to be found in protected bays or harbors relative to wave-swept rocky intertidal sites. This study found that there was a greater number of encrusting bryozoan species found across all sites, with *P. californica* being the only erect species found out of 15 species. Since *P. californica* was the only erect species found at any of the rocky intertidal and harbor sites, there is not enough sampling performed to determine if erect species are truly more prominent in protected harbors than outer rocky coast sites, although it could be inferred that encrusting morphologies are selected for in highly disturbed habitats based on the larger presence of encrusting species on the wave-swept outer coast.

Of the 20 bryozoan samples that were sequenced, this study successfully identified potentially one new species of *Microporella* (BLEED 1897; See Table 2 and Appendix H Figure 8) from Palmer's Point and one new species of *Parasmittina* (BLEED 1219; See Table 2 and Appendix H Figure 9) from Morro Bay that are yet to be fully taxonomically described. These samples were found to belong to the genera *Microporella* and *Parasmittina* respectively but are not the same genetic species as other species identified for each genus in the phylogenies and did not resemble the taxonomic descriptions for other species within each genus. This demonstrates that there are evolutionarily unique species present along the California coast that remain to be discovered. This is not surprising given the overall lack of study of Bryozoa along the California coastline.

Another important species that was found in this study was sample BLEED 1895 (named *Celleporella hyalina* in the results of this study). Although only one genetic sequence was included in the phylogenetic analysis of this study, sample BLEED 1895 had two potential morphological variations identified (described as *Celleporella hyalina* sp. A and *Celleporella hyalina* sp. B in the SEM images- see Appendix H Figure 10) whose only differences noted were a more developed umbo on the autozooids and a different distribution pattern of pseudopores on the ovicells in sp. B in comparison to sp. A. Although sample BLEED 1895 (whose genetic sequence corresponds with the morphology of *C. hyalina* sp. A) formed a fully supported monophyly with the *C. hyalina* sample from NCBI (100 BS/1 PP) in the genetic analysis, other studies suggest the

possibility that BLEED 1895 could be a different morphotype if not subspecies of *C. hyalina* than the sequence included from NCBI.

While *C. hyalina* is known to have a cosmopolitan distribution, studies have found that *C. hyalina* samples from different geographic regions are generally morphologically similar but have produced genetically distinct lineages that are reproductively incompatible with each other, suggesting that *C. hyalina* may be producing cryptic subspecies (Gómez et al., 2007; Hoare et al., 2001; Waeschenbach et al., 2012a). Since this study included only one sample of *C. hyalina*, more research would need to be performed in order to examine the prevalence of the morphological variation seen in our collected samples of *C. hyalina* in northern California and how genetic diversity within this species compares to other global samples of *C. hyalina* (Mackie et al., 2012; Navarrete et al., 2005; Waeschenbach et al., 2012a).

Due to the limited number of sampling sites and sampling periods in this study, it is estimated that an additional 100 bryozoan species at minimum are missing from this phylogenetic and geographic analysis of the California coastline (Soule et al., 2007). While all of the species identified by my study (with the exception of *F. delicia*) are known to be native to the California coastline, species that are known to also be present but were not collected in this study include the invasive species *Watersipora subtorquata* and *Bugula neritina* (Fehlauer-Ale et al., 2014; Mackie et al., 2012; Marraffini et al., 2017). It is recognized that specimens obtained from the rocky outer coast could only be sequenced from two sites north of Cape Mendocino, a biogeographic break for many invertebrate species, while the specimens obtained from harbor sites were all obtained

from sites well south of another biogeographic break in San Francisco Bay. Since there were no harbor sites sampled from northern California, or any rocky intertidal sites with samples sequenced from south of Cape Mendocino, a fully comprehensive survey of the way in which bryozoan diversity varies across the complete coastline as well as differs from bays to outer coast habitats still needs to be completed.

CONCLUSIONS

This study produced 20 new bryozoan mitogenomic sequences and identified 15 distinct species from 10 different families using high-throughput sequencing to form the first comprehensive phylogenomic analysis for multiple bryozoan families along the California coastline. A complete mitogenome including all 15 mitochondrial genes was also produced for 13 out of the 20 bryozoans sequenced which is significant for improving the availability of genetic data for lesser studied taxa. It developed a low computational power bioinformatics pipeline, therefore increasing the accessibility of phylogenomic analysis. Future studies would benefit from increased genetic and taxonomic investigation into the bryozoan biodiversity of the California coastline. The new findings and insights gained from the phylogenetic and preliminary data for phylogeographic analyses performed in this thesis are useful in providing a better understanding of the diversity and distribution of bryozoans throughout the California coastline and can help to infer how the presence of nonindigenous bryozoan species may impact marine invertebrate communities. By continuing to increase the availability of molecular data as well as update morphological descriptions of local bryozoan diversity for intertidal and shallow water depths in California, further data can be integrated with the phylogenies developed in this study to examine more robust evolutionary hypotheses on bryozoan diversity and how their distribution impacts marine invertebrate communities throughout California and the eastern Pacific.

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APPENDICES

Appendix A

Appendix Table 1: Metadata for all cheilostome bryozoans collected in the study that were sequenced at the Norwegian Sequencing Centre. The genus and species names for each taxon are given where available, followed by a BLEED number (Bryozoan Lab for Ecology, Evolution and Development number). The collection site, city, collectors, and collection dates are given as well as descriptions of latitude (Lat) and longitude (Long). Bryozoans collected from Trinidad were collected from the intertidal while bryozoans collected from Long Beach, Morro Bay, Newport, and San Francisco were collected from settlement panels at a depth of 1 meter. Specimens were collected by Hannah Lee, Ismael Chowdhury, Sean Craig, Cody Henrikson, Natasha Hitchcock, Stacey Havard, Lina Ceballos-Osuna, Linda McCann, Andrew Chang, and Michele Repetto. Preservation was either in ethanol or air-dried as indicated. The last column gives information on whether the morphology form of the specimen was erect or encrusting. Species were identified from SEM images by Emanuela Di Martino, Mali H. Ramsfjell, and Linda McCann. Samples that were removed from both the thesis and appendix analyses are listed in Appendix Table 3.

Taxon	BLEED	Site	City	Collected by	Collection Date	Lat	Long	Preservation	Form
Chaperiopsis cf. patula	1889	Baker Beach	Trinidad, CA	H. Lee & I. Chowdhury	11/10/2019	41.0672	-124.1565	Dried	Encrusting
Celleporella sp.	1890	Baker Beach	Trinidad, CA	H. Lee & I. Chowdhury	11/10/2019	41.0672	-124.1565	Dried	Encrusting
Integripelta bilabiata	1891	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Jullienula sp.	1892	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Dendrobeania lichenoides	1893	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Tegella horrida	1894	Palmer's Point	Trinidad, CA	H. Lee & I. Chowdhury	12/9/2019	41.131	-124.1638	95% ethanol	Encrusting

Taxon	BLEED	Site	City	Collected by	Collection Date	Lat	Long	Preservation	Form
Celleporella hyalina	1895	Palmer's Point	Trinidad, CA	H. Lee & I. Chowdhury	12/9/2019	41.131	-124.1638	95% ethanol	Encrusting
Cauloramphus californiensis	1896	Palmer's Point	Trinidad, CA	H. Lee & I. Chowdhury	12/9/2019	41.131	-124.1638	95% ethanol	Encrusting
Microporella sp.	1897	Palmer's Point	Trinidad, CA	H. Lee & I. Chowdhury	12/9/2019	41.131	-124.1638	95% ethanol	Encrusting
Microporella californica	1898	Palmer's Point	Trinidad, CA	H. Lee & I. Chowdhury	12/9/2019	41.131	-124.1638	95% ethanol	Encrusting
Rhynchozoon cf. tumulosum	1899	Palmer's Point	Trinidad, CA	H. Lee & I. Chowdhury	12/9/2019	41.131	-124.1638	95% ethanol	Encrusting
Microporella umbonata	1900	Palmer's Point	Trinidad, CA	H. Lee & I. Chowdhury	12/9/2019	41.131	-124.1638	95% ethanol	Encrusting
Pomocellaria californica	1901	Palmer's Point	Trinidad, CA	H. Lee & I. Chowdhury	12/9/2019	41.131	-124.1638	95% ethanol	Erect
Flustridae	1902	Baker Beach	Trinidad, CA	H. Lee & I. Chowdhury	11/10/2019	41.0672	-124.1565	Dried	Encrusting
Fenestrulina umbonata	1903	Baker Beach	Trinidad, CA	H. Lee & I. Chowdhury	11/10/2019	41.0672	-124.1565	Dried	Encrusting
Copidozoum adamantum	1904	Baker Beach	Trinidad, CA	H. Lee & I. Chowdhury	11/10/2019	41.0672	-124.1565	Dried	Encrusting
Copidozoum adamantum	1905	Baker Beach	Trinidad, CA	H. Lee & C. Henrikson	11/23/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Chaperiopsis patula	1906	Baker Beach	Trinidad, CA	H. Lee & C. Henrikson	11/23/2019	41.0672	-124.1565	200 proof ethanol	Encrusting
Parasmittina collifera	1907	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Microporella catalinensis	1908	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Tegella horrida	1909	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Copidozoum adamantum	1910	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting

Taxon	BLEED	Site	City	Collected by	Collection Date	Lat	Long	Preservation	Form
Aetea sp.	1911	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Erect
Pomocellaria californica	1912	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Erect
Dendrobeania lichenoides	1913	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Tegella horrida	1914	Baker Beach	Trinidad, CA	S. Craig & C. Henrikson	11/24/2019	41.04919	-124.1279	200 proof ethanol	Encrusting
Thalamoporella californica	1211	Cabrillo Marina	Long Beach, CA	N. Hitchcock	2017	33.718	-118.278	95% ethanol	Encrusting
Primavelans insculpta	1214	City Harbor	Morro Bay, CA	S. Havard	8/27/2013	35.371	-120.858	95% ethanol	Encrusting
Parasmittina collifera	1216	201 Main	Morro Bay, CA	S. Havard	2013	35.356	-120.847	95% ethanol	Encrusting
Parasmittina sp.	1219	Launch Ramp	Morro Bay, CA	S. Havard	9/5/2013	35.358	-120.851	95% ethanol	Encrusting
Schizoporella occidentalae	1220	Harbor Patrol Public Dock	Newport, CA	L. Ceballos	2017	33.6067	-117.93	95% ethanol	Encrusting
Aetea pseudoanguina	1224	Tidelands	Morro Bay, CA	L. McCann	9/4/2013	35.36	-120.852	95% ethanol	Erect
Smittoidea prolifica	1239	Loch Lomond Marina	San Francisco, CA	L. Ceballos	2013	37.972	-122.482	95% ethanol	Encrusting
Fenestrulina delicia	1244	201 Main	Morro Bay, CA	A. Chang	8/30/2013	35.356	-120.847	95% ethanol	Encrusting
Pomocellaria californica	1246	City Harbor	Morro Bay, CA	M. Repetto	8/27/2013	35.371	-120.858	95% ethanol	Erect

Appendix B

Appendix Table 2: All taxa generated and analyzed in this study. The family, genus, and species (if known) are given for each specimen sequenced at the Norwegian Sequencing Centre followed by the corresponding BLEED number (Bryozoan Lab for Ecology, Evolution and Development) and NCBI accession number. BLEED numbers marked with an * indicate species that were not previously sequenced prior to this study. The size, in base pairs (bp), of the mitogenome (Mt) are only shown if it is complete/circularized, with NO indicating that a complete mitogenome was not formed. The SEM images for each specimen are available in under the corresponding figure number in Appendix H. BLEED samples 1889, 1893, 1905, and 1239 were removed from the main phylogenetic analysis in the thesis due to low DNA quality but are included in the appendix analyses.

Family	Genus	Species	BLEED	Accession nbr	Mt size (bp)	SEM
Chaperiidae	*Chaperiopsis*	*cf. patula*	1889*	OK244803	NO	App. Fig. 26
Bugulidae	*Dendrobeania*	*lichenoides*	1893	OK245241	NO	App. Fig. 27
Calloporidae	*Tegella*	*horrida*	1894*	OK245189, OK245190, OK245191	NO	App. Fig. 11
Hippothoidae	*Celleporella*	*hyalina*	1895	OK244797, OK244798	NO	App. Fig. 10
Calloporidae	*Cauloramphus*	*californiensis*	1896*	OK244778	14,573	App. Fig. 28
Microporellidae	*Microporella*	*sp.*	1897*	OK245155	13,563	App. Fig. 8
Phidoloporidae	*Rhynchozoon*	*cf. tumulosum*	1899	OK244996	14,102	App. Fig. 20
Microporellidae	*Microporella*	*umbonata*	1900*	OK245161	13,931	App. Fig. 22
Candidae	*Pomocellaria*	*californica*	1901	OK244975	15,733	App. Fig. 14
Fenestrulinidae	*Fenestrulina*	*umbonata*	1903*	OK244859	14,780	App. Fig. 23
Calloporidae	*Copidozoum*	*adamantum*	1905	OK245119, OK245120, OK245121	NO	App. Fig. 29
Chaperiidae	*Chaperiopsis*	*patula*	1906*	OK244804	NO	App. Fig. 25
Smittinidae	*Parasmittina*	*collifera*	1907*	OK244944	14,299	App. Fig. 17

Family	Genus	Species	BLEED	Accession nbr	Mt size (bp)	SEM
Calloporidae	*Tegella*	*horrida*	1909*	OK245192, OK245193, OK245194	NO	App. Fig. 12
Calloporidae	*Copidozoum*	*adamantum*	1910	OK244819	NO	App. Fig. 30
Candidae	*Pomocellaria*	*californica*	1912	OK244976	15,733	App. Fig. 15
Calloporidae	*Tegella*	*horrida*	1914*	OK245195, OK245196, OK245197	NO	App. Fig. 13
Thalamoporellidae	*Thalamoporella*	*californica*	1211	OK245202	13,928	App. Fig. 31
Pacificincolidae	*Primavelans*	*insculpta*	1214*	OK244984	17,268	App. Fig. 21
Smittinidae	*Parasmittina*	*collifera*	1216*	OK244952	14,305	App. Fig. 18
Smittinidae	*Parasmittina*	*sp.*	1219*	OK244953	14,302	App. Fig. 9
Smittinidae	*Smittoidea*	*prolifica*	1239	OK245026	NO	App. Fig. 19
Fenestrulinidae	*Fenestrulina*	*delicia*	1244*	OK244853	NO	App. Fig. 24
Candidae	*Pomocellaria*	*californica*	1246	OK244974	15,733	App. Fig. 16

Appendix C

Appendix Table 3: All samples that were removed from both the thesis and appendix analyses. The taxon name, BLEED number (Bryozoan Lab for Ecology, Evolution and Development), NCBI accession number (if applicable), location, and reason for removal from analyses are listed below. The size, in base pairs (bp), of the complete/circularized mitogenome (NO indicates that a complete mitogenome was not formed) and the number of genes produced are given if applicable.

Taxon	BLEED	Accession nbr	Location	Reason for Removal	Mt size (bp)	Genes
Celleporella sp.	1890	NA	Baker Beach	Correct genes were not recovered.	NO	0
Integripelta bilabiata	1891	OK244871	Baker Beach	SEM voucher ID did not agree with phylogenetic ID.	NO	1
Jullienula sp.	1892	NA	Baker Beach	SEM voucher ID did not agree with phylogenetic ID.	17,937	15
Microporella californica	1898	NA	Palmer's Point	SEM voucher ID did not agree with phylogenetic ID.	NO	12
Flustridae	1902	NA	Baker Beach	Correct genes were not recovered.	NO	0
Copidozoum adamantum	1904	OK245118	Baker Beach	SEM voucher ID did not agree with phylogenetic ID.	NO	12
Microporella catalinensis	1908	NA	Baker Beach	No reads produced.	NO	NA
Aetea sp.	1911	NA	Baker Beach	SEM voucher ID did not agree with phylogenetic ID, ctenostome contaminant.	NO	15
Dendrobeania lichenoides	1913	OK244827	Baker Beach	Correct genes were not recovered.	NO	0
Schizoporella occidentalae	1220	OK245011	Newport	SEM voucher ID did not agree with phylogenetic ID.	NO	2
Aetea pseudoanguina	1224	NA	Morro Bay	SEM voucher ID did not agree with phylogenetic ID.	14,309	13

Appendix D

Appendix Table 4: The number of genes used in phylogenetic reconstruction for the bryozoan cheilostome sequences generated and analyzed in this study. The genes that were available are stated as available (1) or unavailable (0) in the following columns. See Appendix B for NCBI accession numbers.

Taxon	BLEED	rrnL	rrnS	atp6	atp8	cox1	cox2	cox3	cob	nad1	nad2	nad3	nad4	nad4l	nad5	nad6	Total Genes
Chaperiopsis cf. patula	1889	0	0	0	1	1	1	0	1	0	1	1	1	1	0	0	8
Dendrobeania lichenoides	1893	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tegella horrida	1894	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Celleporella sp.	1895	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Cauloramphus californiensis	1896	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Microporella sp.	1897	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	14
Rhynchozoon cf. tumulosum	1899	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	14
Microporella umbonata	1900	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	14
Pomocellaria californica	1901	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Fenestrulina umbonata	1903	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	14
Copidozoum adamantum	1905	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
Chaperiopsis	1906	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15

Taxon	BLEED	rrnL	rrnS	atp6	atp8	cox1	cox2	cox3	cob	nad1	nad2	nad3	nad4	nad4l	nad5	nad6	Total Genes
patula																	
Parasmittina collifera	1907	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Tegella horrida	1909	1	1	1	1	1	1	0	1	0	1	1	1	1	1	0	12
Copidozoum adamantum	1910	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	13
Pomocellaria californica	1912	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Tegella horrida	1914	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Thalamoporella californica	1211	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	14
Primavelans insculpta	1214	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	14
Parasmittina collifera	1216	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Parasmittina sp.	1219	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Smittoidea prolifica	1239	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Fenestrulina delicia	1244	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Pomocellaria californica	1246	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15

Appendix E

Appendix Table 5: The bryozoan cheilostome and ctenostome sequences used from NCBI are cited with their accession numbers. The BLEED number (Bryozoan Lab for Ecology, Evolution and Development) is given if applicable. The collection site location and the depth (in meters) are given if available.

Family	Genus	Species	Accession nbr	BLEED	Location	Depth (m)
Alcyonidiidae	*Alcyonidioides*	*mytili*	JN681069, JN681102, AEV21493, AEV21531		Unavailable	Unavailable
Vesiculariidae	*Amathia*	*citrina*	KM373503, JN681121		Ferrol, San Felipe, Spain	Unavailable
Nolellidae	*Anguinella*	*palmata*	JN681101, AJB84768, AEV21530		Parana, Ilha do Mel, Morro do Sabao, Brazil	Unavailable
Hippothoidae	*Antarctothoa*	*delta*	MT293076	703	Steward Island, New Zealand	0
Arachnopusiidae	*Arachnopusia*	*unicornis*	MT293085	221	New Zealand	47
Bugulidae	*Bicellariella*	*ciliata*	MT293086	560	Off Kristineberg, Sweden	15-45
Calloporidae	*Callopora*	*lineata*	JN681080, AEV21506, AEV21540		Unavailable	Unavailable
Hippothoidae	*Celleporella*	*hyalina*	NC_018344		Vatlestraumen, Bergen, Norway; Church Island, Menai Strait, Wales, UK	0

Family	Genus	Species	Accession nbr	BLEED	Location	Depth (m)
Candidae	*Cradoscrupocellaria*	*reptans*	MT293075	1192	Bergen, Norway	Unknown
Fenestrulinidae	*Fenestrulina*	*malusii*	MG977059, MG977074, MG977105, MG977128	17	Scapa Flow, Orkney Island, Scotland	28
Fenestrulinidae	*Fenestrulina*	*sp. nov. 1*	MG977057, MG977073, MG977106, MG977121	148	Allans Beach, Dunedin, New Zealand	0
Flustrellidridae	*Flustrellidra*	*hispida*	NC_008192		Mumbles and Aberystwyth, Wales, UK	Unknown
Microporellidae	*Microporella*	*cf. ciliata*	MG977064, MG977079, AVV48237, AVV48243		Unavailable	Unavailable
Microporellidae	*Microporella*	*sp.*	MG977063, MG977078, AVV48236	387	Qingdao, China	0
Microporellidae	*Microporella*	*sp. nov. 2*	MG977075, MG977091, MG977103, MG977126	11	off Dunedin, New Zealand	88
Paludicellidae	*Paludicella*	*sp.*	JN681070, JN681103, AEV21494, AEV21532		Unavailable	Unavailable
Smittinidae	*Parasmittina*	*aotea*	MT293094	86	New Zealand	Unknown
Smittinidae	*Parasmittina*	*jeffreysi*	MT293102	1202	Arctic	146-149
Smittinidae	*Parasmittina*	*solenosmilioides*	MT293113	1267	New Zealand	0

Family	Genus	Species	Accession nbr	BLEED	Location	Depth (m)
Chaperiidae	*Patsyella*	*acanthodes*	MT293109	131	New Zealand	Unknown
Rhabdozoidae	*Rhabdozoum*	*wilsoni*	MT293111	695	off White Rock, Stewart Island, New Zealand	72
Phidoloporidae	*Rhynchozoon*	*angulatum*	MT293088	694	off White Rock, Stewart Island, New Zealand	72
Phidoloporidae	*Rhynchozoon*	*zealandicum*	MT293099	127	New Zealand	0-1
Scruparidae	*Scruparia*	*chelata*	JN681081, JN681115, AEV21507, AEV21541		Unavailable	Unavailable
Steginoporellidae	*Steginoporella*	*perplexa*	MT293100	100	New Zealand	170
Phidoloporidae	*Stephanollona*	*scintillans*	MT293120	679	New Owen Island, New Zealand	77
Watersiporidae	*Watersipora*	*subtorquata*	NC011820		Qingdao Huiquan Beach, Qingdao, China	0

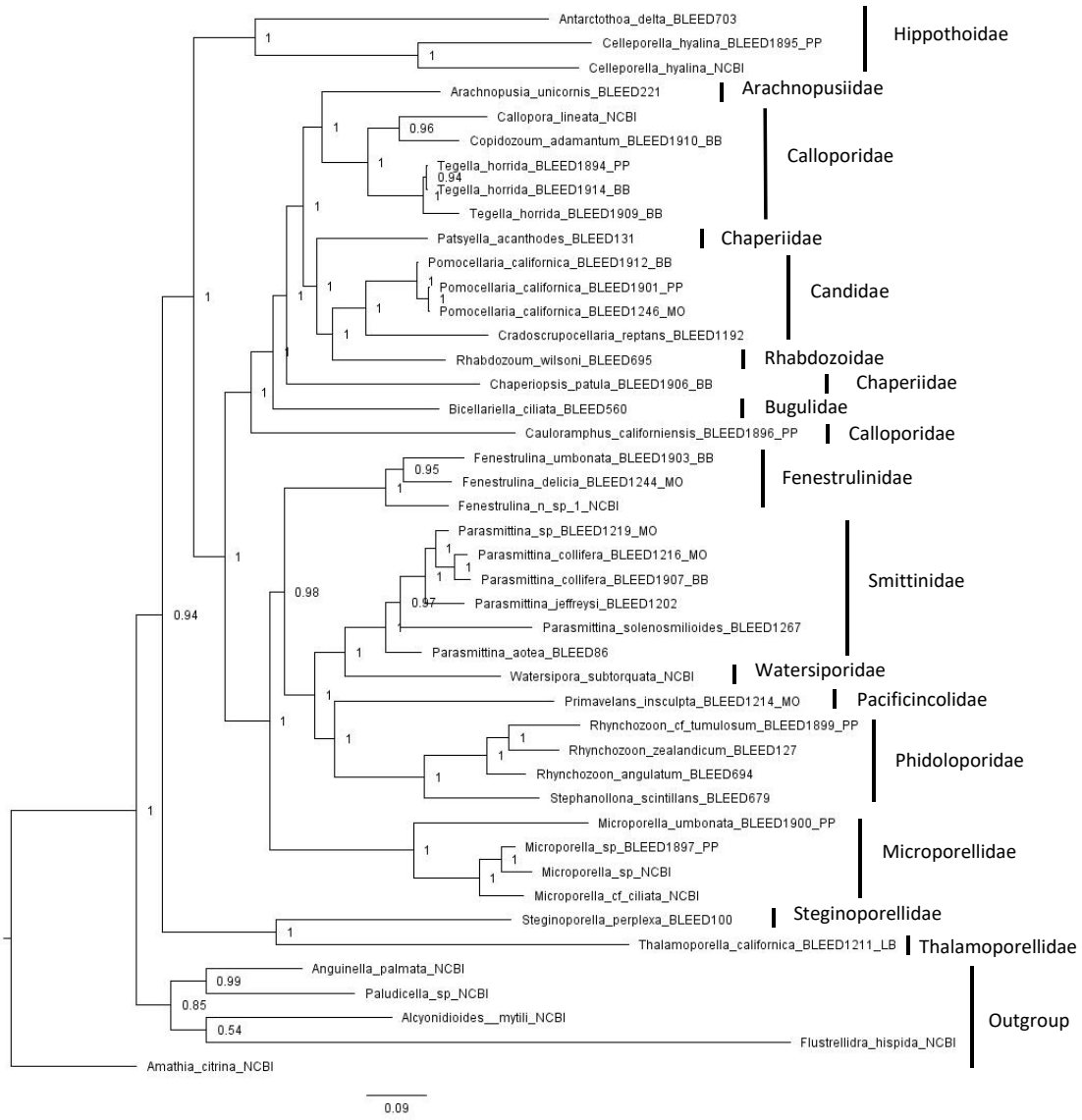
Appendix Table 6: The number of genes used in phylogenetic reconstruction for the bryozoan cheilostome and ctenostome sequences from NCBI are summarized. The genes that were available are stated as available (1) or unavailable (0) in the following columns. See Appendix Table 5 for NCBI accession numbers.

Taxon from NCBI	rrnL	rrnS	atp6	atp8	cox1	cox2	cox3	cob	nad1	nad2	nad3	nad4	nad4l	nad5	nad6	Total Genes
Alcyonidioides mytili	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	5
Amathia citrina	1	1	0	0	1	0	0	1	0	0	0	0	0	0	0	4
Anguinella palmata	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	4
Antarctothoa delta	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	12
Arachnopusia unicornis	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Bicellariella ciliata	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Callopora lineata	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	4
Celleporella hyalina	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	14
Cradoscrupocellaria reptans	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	14
Fenestrulina malusii	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	4
Fenestrulina sp. n. 1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	4
Flustrellidra hispida	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	13
Microporella cf. ciliata	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	5
Microporella sp.	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	5
Microporella sp. n. 2	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	4
Paludicella sp.	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	5

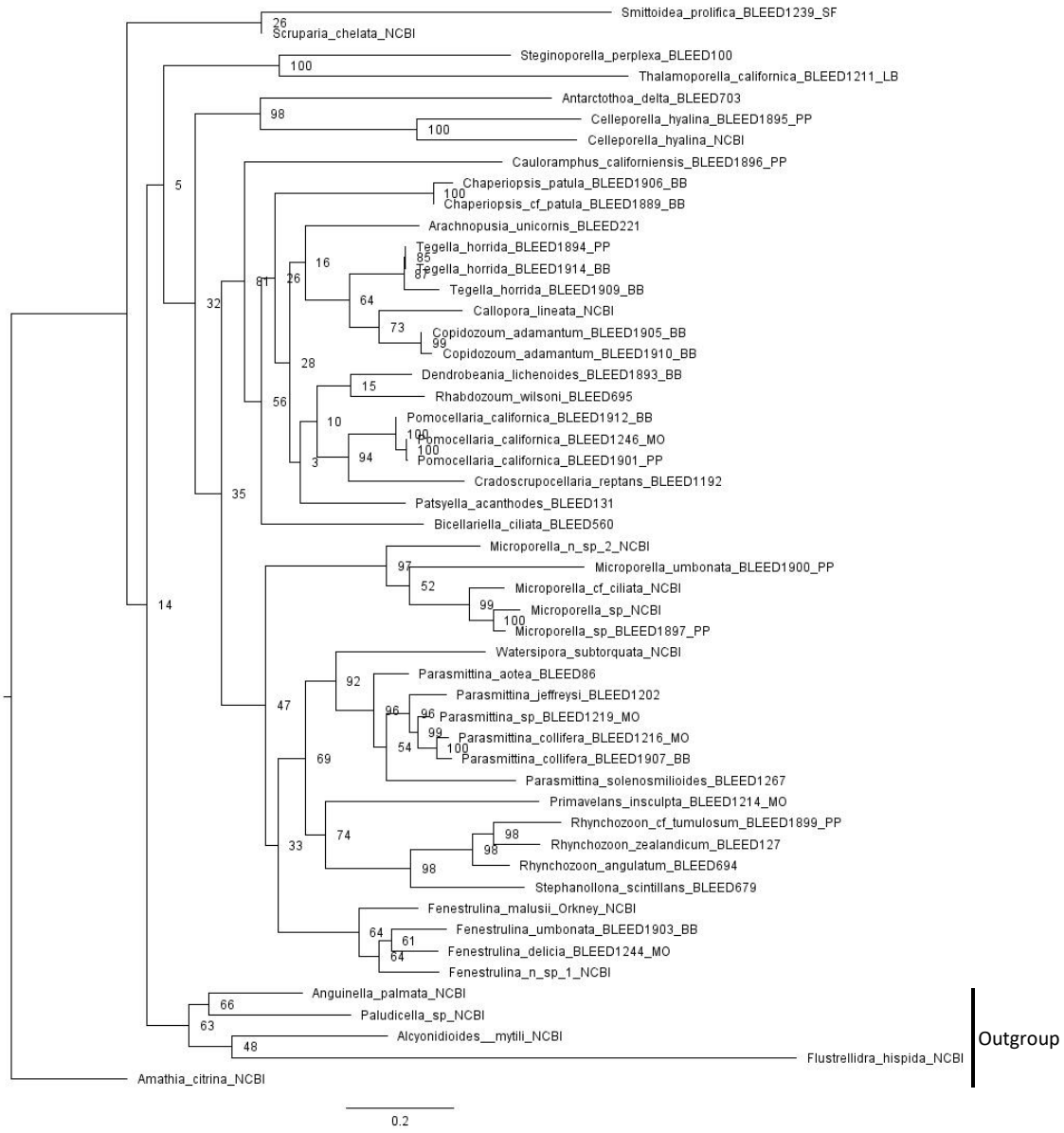
Taxon from NCBI	rrnL	rrnS	atp6	atp8	cox1	cox2	cox3	cob	nad1	nad2	nad3	nad4	nad4l	nad5	nad6	Total Genes
Parasmittina aotea	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	13
Parasmittina jeffreysi	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Parasmittina solenosmilioides	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Patsyella acanthodes	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	14
Rhabdozoum wilsoni	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	15
Rhynchozoon angulatum	1	1	1	0	1	1	1	1	1	1	0	1	1	1	1	13
Rhynchozoon zealandicum	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	12
Scruparia chelata	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	5
Steginoporella perplexa	0	1	1	0	1	1	1	1	1	1	0	1	1	1	1	12
Stephanollona scintillans	1	0	1	0	1	1	1	1	1	1	0	1	1	1	1	12
Watersipora subtorquata	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	13

Appendix F

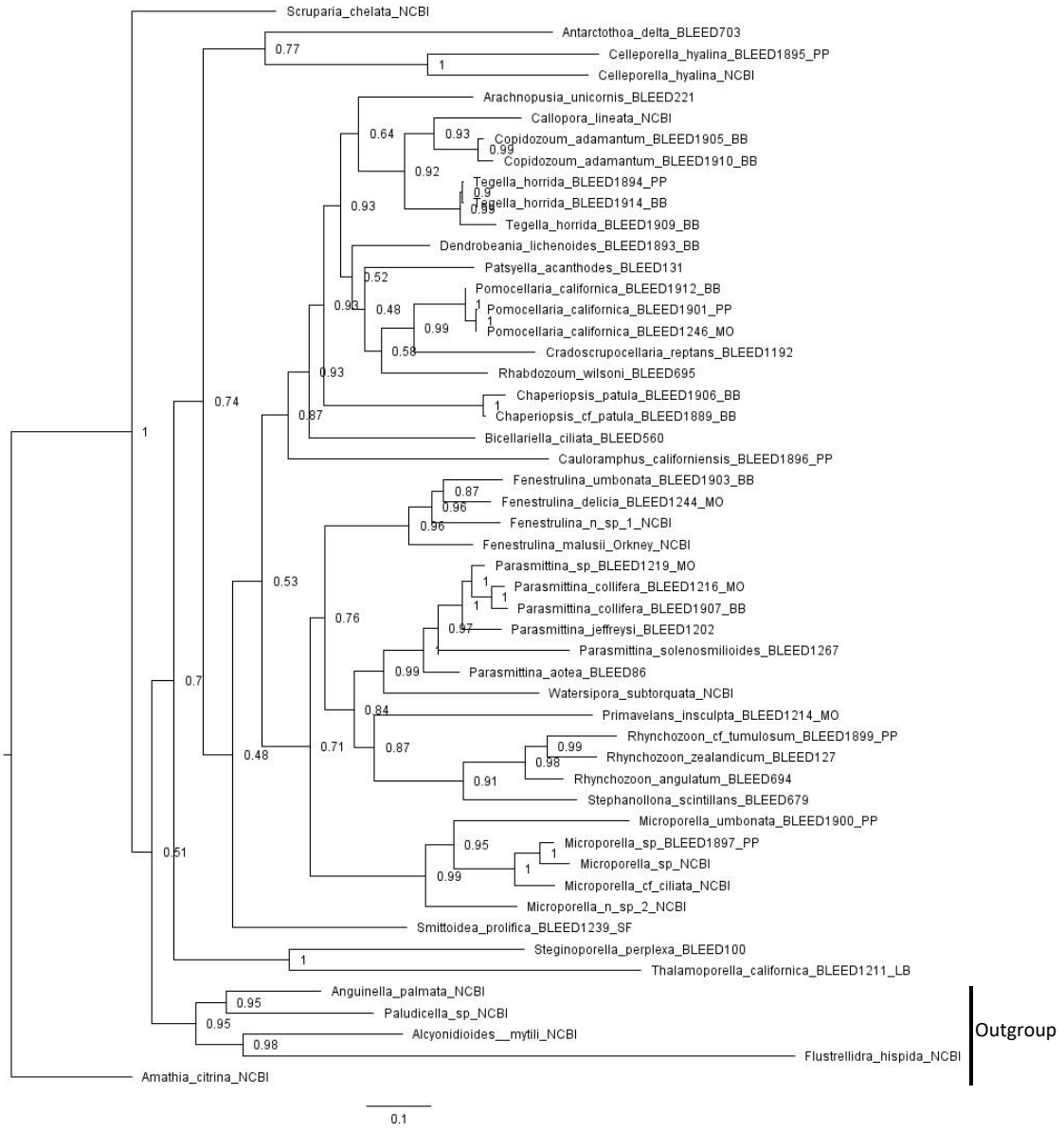
Phylogenetic trees were generated in RAxML v.8.2.12 (Stamatakis, 2014) for maximum likelihood (ML) analyses and in MrBayes v.3.2.7a (Huelsenbeck & Ronquist, 2001; Ronquist et al., 2012) for Bayesian inference (BI). The GTR model was used for the two rRNA genes (rrnL and rrnS) and the MtZoa model was used for the protein-coding genes in the ML analyses. The mtREV model was used for all BI analyses. Taxa with unstable phylogenetic affinities were identified and removed using MESQUITE v.3.61 only if morphological identification did not agree with phylogenetic placement or if sequences were unable to be produced. BLEED samples 1889, 1893, 1905, and 1239 were removed from the main phylogenetic analysis in the thesis due to low DNA quality but have been included in the appendix analyses (Appendix Figures 2-7). Sample BLEED 1239 adds one additional collection site from San Francisco to the dataset. Maximum likelihood and Bayesian inference trees were produced based on the full concatenated appendix dataset as well as on geographic habitat type (rocky intertidal sites or harbor sites).



Appendix Figure 1: The phylogeny of cheilostome bryozoans collected along the California coastline between Trinidad, CA and Long Beach, CA based on 15 mitochondrial genes. Bayesian topology of 39 ingroup taxa (20 taxa from this study) and 5 ctenostome outgroup taxa with 4,007 nucleotide and amino acid characters inferred using RAxML (100 heuristic searches and bootstrap values from 500 pseudoreplicates). The numbers on the internal nodes are posterior probabilities values (MrBayes). Branches with samples sequenced in this study are labeled with the site abbreviation from which they were collected (PP = Palmer's Point, BB = Baker Beach, MO = Morro Bay and LB = Long Beach). Scale bar indicates number of substitutions per site. Includes the same taxa that are in the maximum likelihood phylogeny in Figure 2 of the results in the main text.



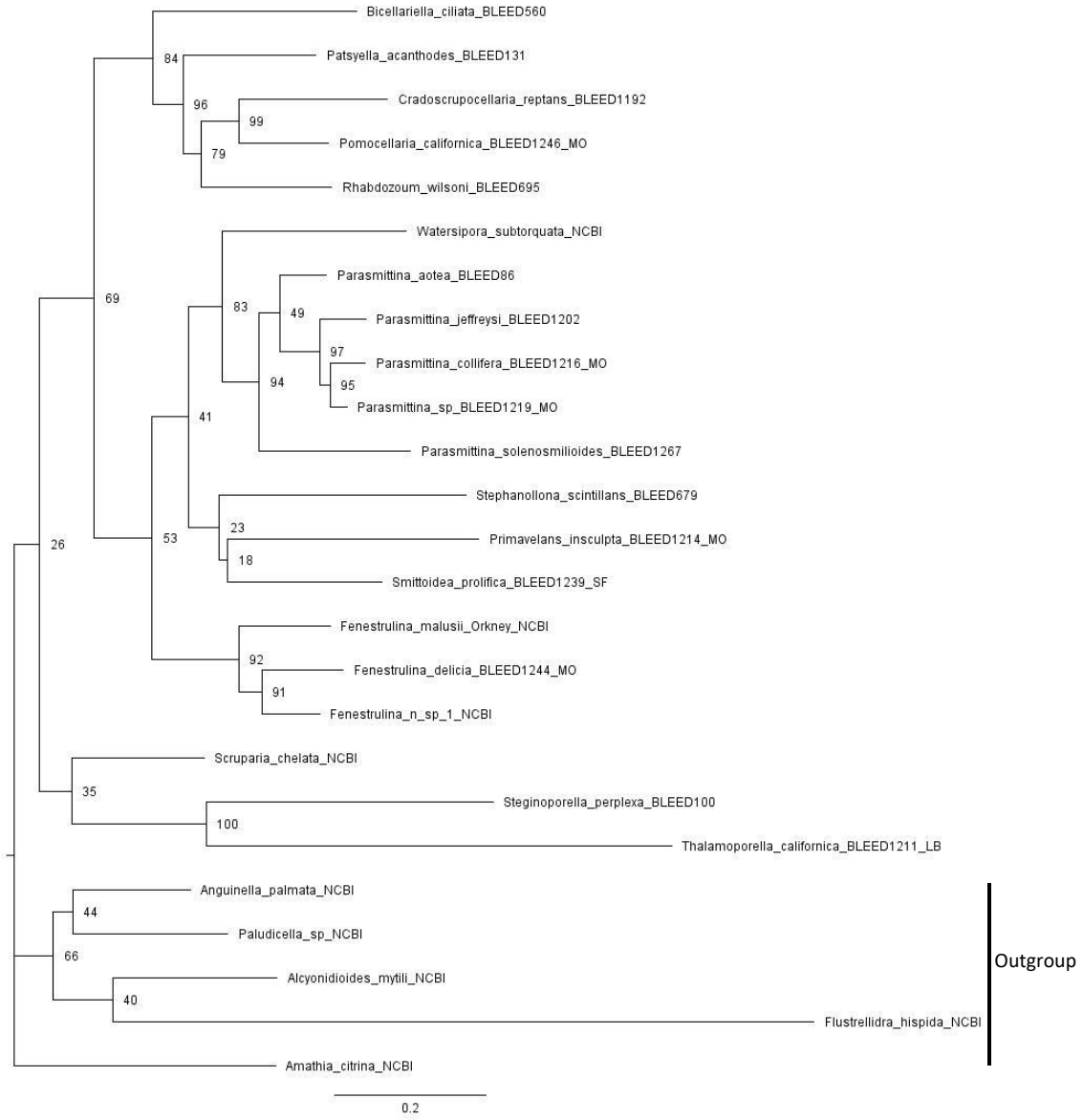
Appendix Figure 2: The phylogeny of cheilostome bryozoans collected along the California coastline between Trinidad, CA and Long Beach, CA based on 15 mitochondrial genes. Maximum likelihood topology of 46 ingroup taxa (24 taxa from this study) and 5 ctenostome outgroup taxa with 4,007 nucleotide and amino acid characters inferred using RAxML (100 heuristic searches and bootstrap of 500 pseudoreplicates). The numbers on the internal nodes are ML bootstrap values (RAxML). Branches with newly sequenced samples are labeled with the site abbreviation from which they were collected (PP = Palmer's Point, BB = Baker Beach, SF = San Francisco Bay, MO = Morro Bay and LB = Long Beach). Scale bar indicates number of substitutions per site. Includes taxa that were removed from the thesis phylogenies for having >70% missing characters (nucleotide or amino acid).



Appendix Figure 3: The phylogeny of cheilostome bryozoans collected along the California coastline between Trinidad, CA and Long Beach, CA based on 15 mitochondrial genes. Bayesian topology of 46 ingroup taxa (24 taxa from this study) and 5 ctenostome outgroup taxa with 4,007 nucleotide and amino acid characters inferred using MrBayes. The numbers on the internal nodes are posterior probability values (MrBayes). Branches with newly sequenced samples are labeled with the site abbreviation from which they were collected (PP = Palmer's Point, BB = Baker Beach, SF = San Francisco Bay, MO = Morro Bay and LB = Long Beach). Scale bar indicates number of substitutions per site. Includes taxa that were removed from the thesis phylogenies for having >70% missing characters (nucleotide or amino acid).

Appendix Figure 4: The phylogeny of cheilostome bryozoans from the rocky outer coast in Humboldt County, CA based on 15 mitochondrial genes. Maximum likelihood topology of 36 ingroup taxa (17 taxa from this study) and 5 ctenostome outgroup taxa with 4,007 nucleotide and amino acid characters inferred using RAxML (100 heuristic searches and bootstrap of 500 pseudoreplicates). The numbers on the internal nodes are ML bootstrap values (RAxML). Branches with newly sequenced samples are labeled with the site abbreviation from which they were collected (PP = Palmer's Point and BB = Baker Beach). Scale bar indicates number of substitutions per site. Includes taxa that were removed from the thesis phylogenies for having >70% missing characters (nucleotide or amino acid).

Appendix Figure 5: The phylogeny of cheilostome bryozoans from the rocky outer coast in Humboldt County, CA based on 15 mitochondrial genes. Bayesian topology of 36 ingroup taxa (17 taxa from this study) and 5 ctenostome outgroup taxa with 4,007 nucleotide and amino acid characters inferred using MrBayes. The numbers on the internal nodes are posterior probabilities (MrBayes). Branches with newly sequenced samples are labeled with the site abbreviation from which they were collected (PP = Palmer's Point and BB = Baker Beach). Scale bar indicates number of substitutions per site. Includes taxa that were removed from the thesis phylogenies for having >70% missing characters (nucleotide or amino acid).



Appendix Figure 6: The phylogeny of cheilostome bryozoans collected from harbors in central and southern California based on 15 mitochondrial genes. Maximum likelihood topology of 20 ingroup taxa (7 taxa from this study) and 5 ctenostome outgroup taxa with 4,116 nucleotide and amino acid characters inferred using RAxML (100 heuristic searches and bootstrap of 500 pseudoreplicates). The numbers on the internal nodes are ML bootstrap values (RAxML). Branches with newly sequenced samples are labeled with the site abbreviation from which they were collected (MO = Morro Bay, LB = Long Beach, and SF = San Francisco Bay). Scale bar indicates number of substitutions per site. Includes taxa that were removed from the thesis phylogenies for having >70% missing characters (nucleotide or amino acid).

Appendix Figure 7: The phylogeny of cheilostome bryozoans collected from harbors in central and southern California based on 15 mitochondrial genes. Bayesian topology of 20 ingroup taxa (7 taxa from this study) and 5 ctenostome outgroup taxa with 4,116 nucleotide and amino acid characters inferred using MrBayes. The numbers on the internal nodes are posterior probabilities (MrBayes). Branches with newly sequenced samples are labeled with the site abbreviation from which they were collected (MO = Morro Bay, LB = Long Beach, and SF = San Francisco Bay). Scale bar indicates number of substitutions per site. Includes taxa that were removed from the thesis phylogenies for having >70% missing characters (nucleotide or amino acid).

Appendix G

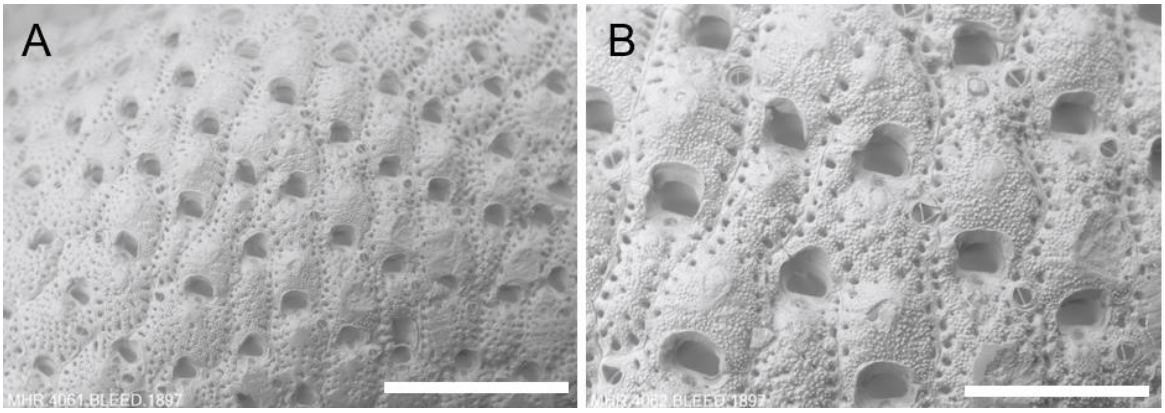
For species that had representatives from multiple rocky outer coast and/or protected harbor sites, the geographic and genetic distances were estimated between each of the representatives within a species to infer potential correlations between the genetic and geographic population structure due to isolation by distance. Geographic distances were estimated between the site coordinates for each individual sample of a given species in kilometers (km). Genetic pairwise distances were estimated using the p-distance model in MEGA X by dividing the observed number of nucleotide and amino acid differences by the total number of sites being compared between two sequences (Kumar et al., 2018). The p-distance model assumes that the higher the p-distance value, the greater the amount of genetic divergence has occurred between two sequences. However, the model is unable to correct for multiple nucleotide or amino acid substitutions at the same site or the differences in evolutionary rates among sites (Strimmer & von Haeseler, 2009). The rate variation among sites was assumed to be a uniform rate for each site and was performed with 500 bootstrap replications. All ambiguous positions were removed for each sequence pair with the pairwise deletion option which excludes a character only when it is absent in one of the two sequences being compared. This maximizes the use of informative character changes at each site but could lead to the over or underestimation of genetic distance since different nucleotide or amino acid regions evolve at different rates. The average genetic distance calculated between species was 3.8% in *T. horrida*, 3.8% in *P. collifera*, and 1.1% in *P. californica*.

Appendix Table 7: Estimates of geographic and evolutionary distances between species with individuals at multiple rocky intertidal and/or harbor sites. The family, species, and individual BLEED number is given for each sample in each comparison. The number of genes used in each comparison are given for each respective sample (see Appendix D for gene availability). The end of each sample ID is labeled with the site abbreviation from which the sample was collected from (PP = Palmer's Point, BB = Baker Beach, and MO = Morro Bay). Samples collected at Palmer's Point and Baker Beach were on the rocky outer coast while those collected at Morro Bay were from within the harbor. The geographic distance between each site is given in kilometers (km). The genetic distance among samples is calculated using the p-distance model with a uniform rate variation. The standard error estimates are given for the genetic distances.

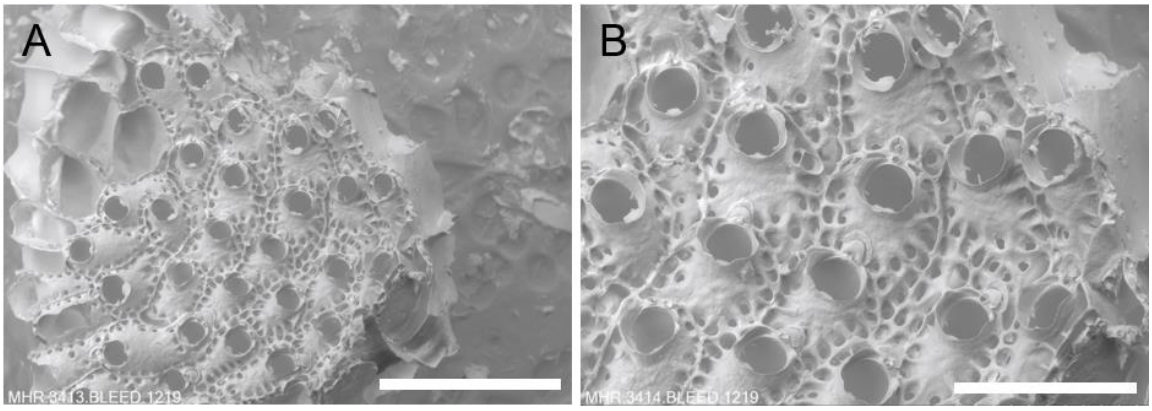
Family	Species	Samples	Genes	Geographic Distance (km)	Genetic Distance	Genetic Std Error
Calloporidae	*Tegella horrida*	BLEED 1894_PP vs BLEED 1909_BB	15 vs 12	9.67629	0.05743	0.00640
		BLEED 1894_PP vs BLEED 1914_BB	15 vs 15	9.67629	0.00108	0.00072
		BLEED 1909_BB vs BLEED 1914_BB	12 vs 15	0	0.05819	0.00639
Smittinidae	*Parasmittina collifera*	BLEED 1907_BB vs BLEED 1216_MO	15 vs 15	694.21540	0.03751	0.00314
Candidae	*Pomocellaria californica*	BLEED 1901_PP vs BLEED 1912_BB	15 vs 15	9.67629	0.01524	0.00190
		BLEED 1901_PP vs BLEED 1246_MO	15 vs 15	701.90702	0.00118	0.0058
		BLEED 1912_BB vs BLEED 1246_MO	15 vs 15	692.29005	0.01686	0.00213

Appendix H

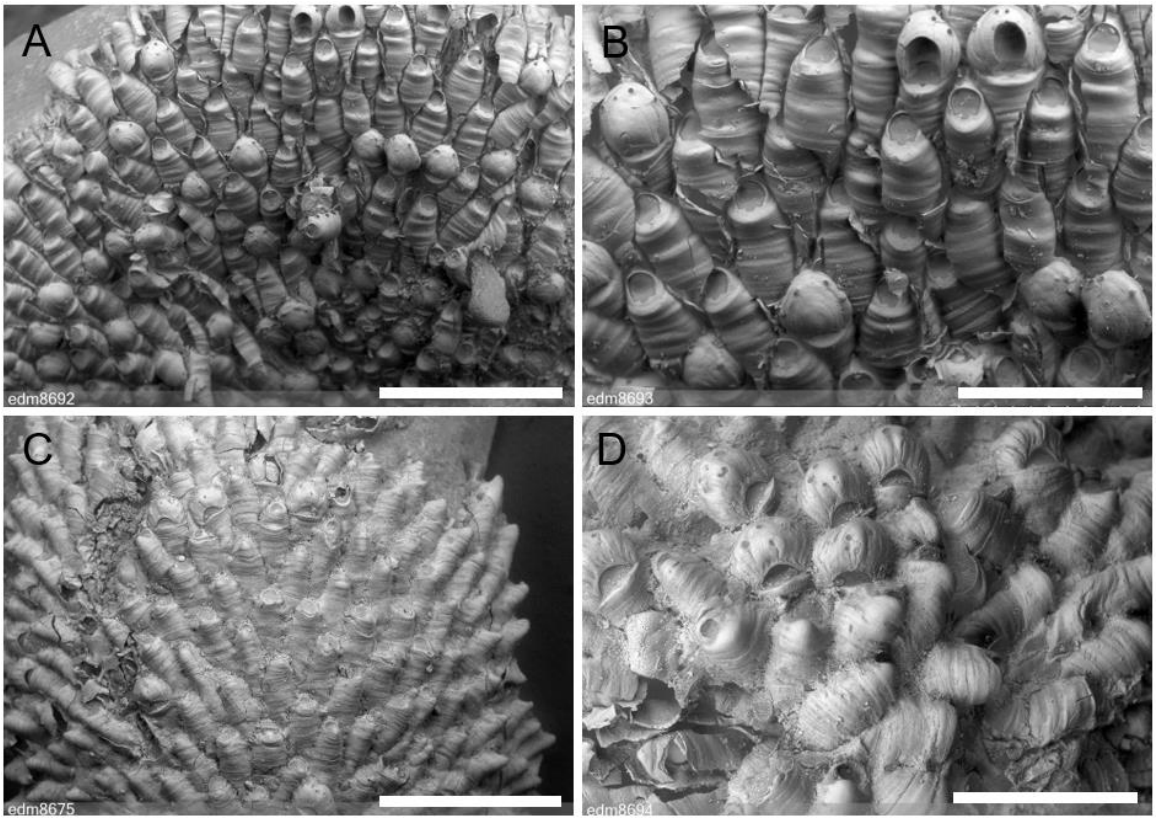
Scanning electron micrograph (SEM) images were taken at the Natural History Museum at the University of Oslo, Oslo, Norway for morphological species identification. The SEM images of all newly sequenced samples in this study that were used in either the main phylogenetic analyses or in the appendix are included as follows.



Appendix Figure 8: *Microporella* sp., BLEED 1897, Palmer's Point, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.

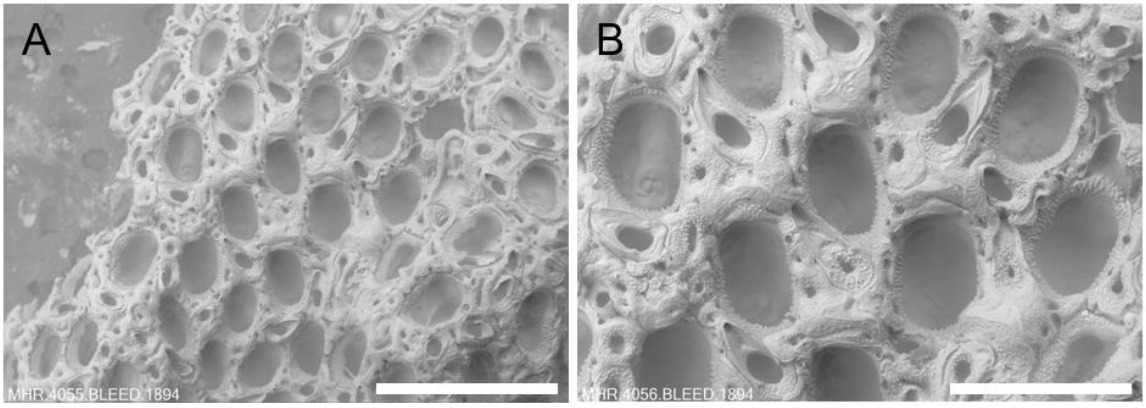


Appendix Figure 9: *Parasmittina* sp., BLEED 1219, Smithsonian Collection Number 301760, Morro Bay, California, USA. Depth: 1 m. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.

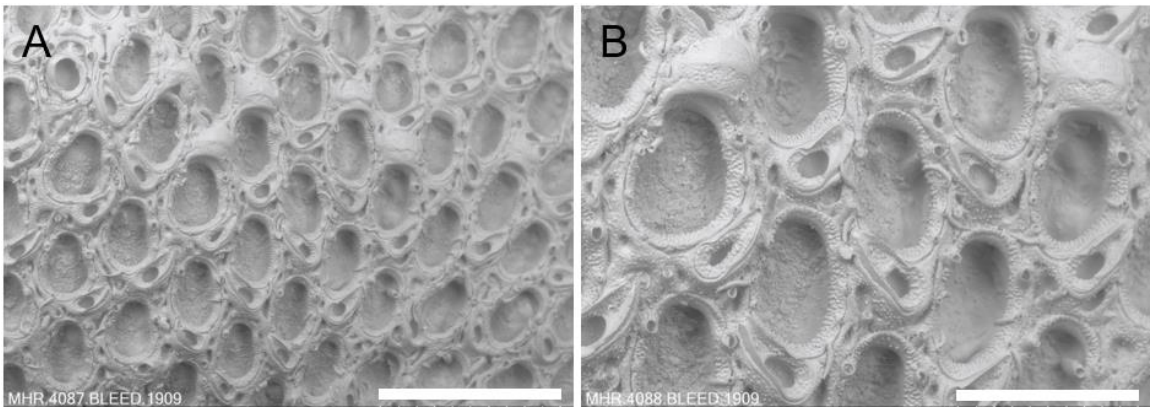


Appendix Figure 10: *Celleporella hyalina sp. A* **(A, B)** and *Celleporella hyalina sp. B* **(C, D)**, BLEED 1895, Palmer's Point, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A, C)** 1 mm; **(B, D)** 500 µm. Photo Credit: Emanuela Di Martino.

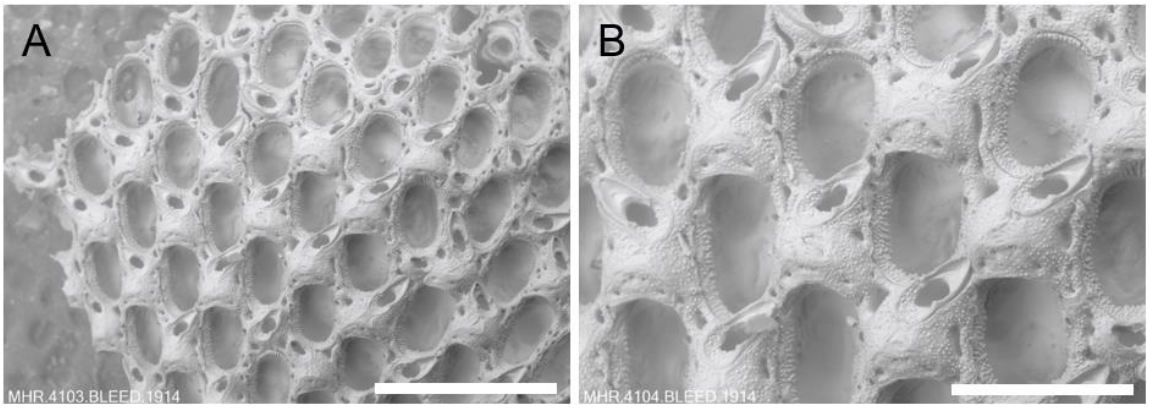
Note: No obvious morphological differences between sp. A and sp. B except for a more developed umbo on the autozooids on sp. B and different distribution pattern of pseudopores on ovicell.



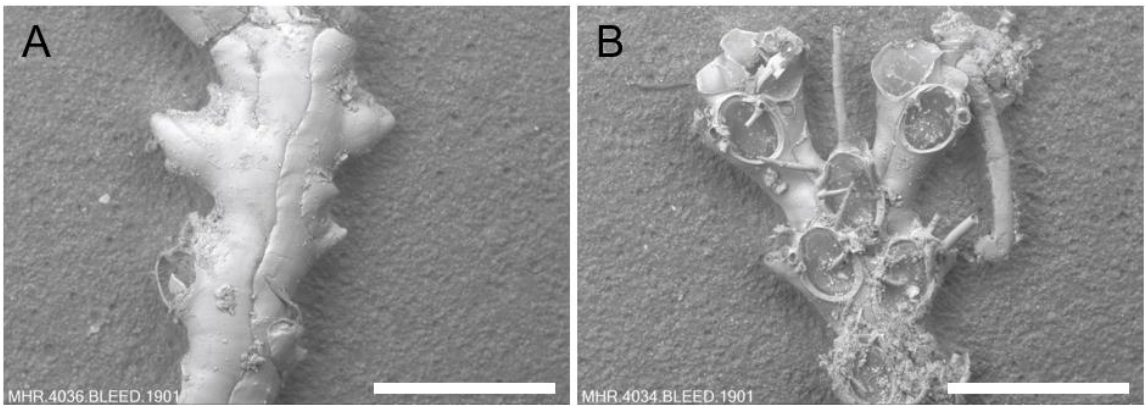
Appendix Figure 11: *Tegella horrida,* BLEED 1894, Palmer's Point, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



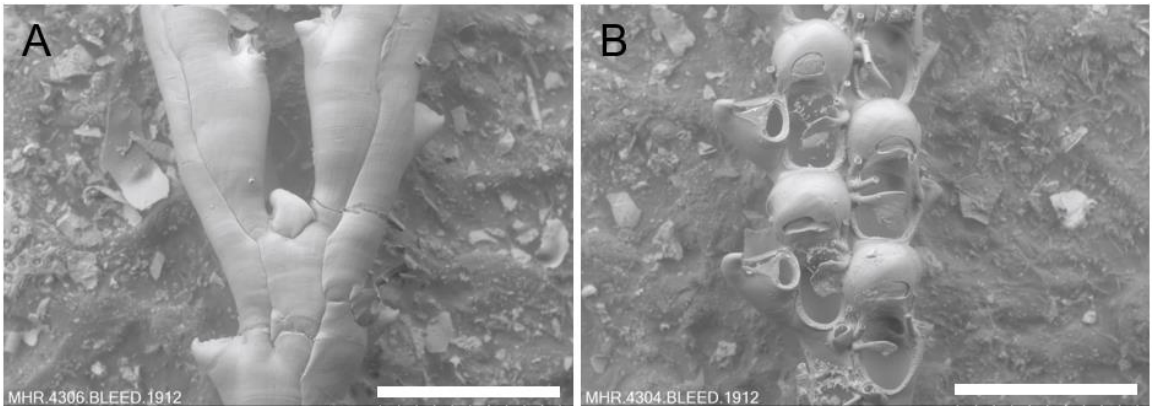
Appendix Figure 12: *Tegella horrida,* BLEED 1909, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



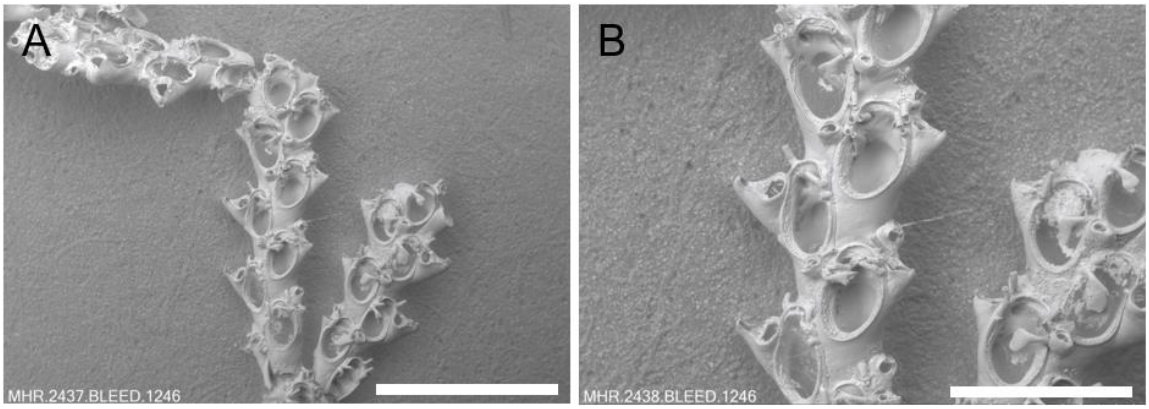
Appendix Figure 13: *Tegella horrida,* BLEED 1914, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



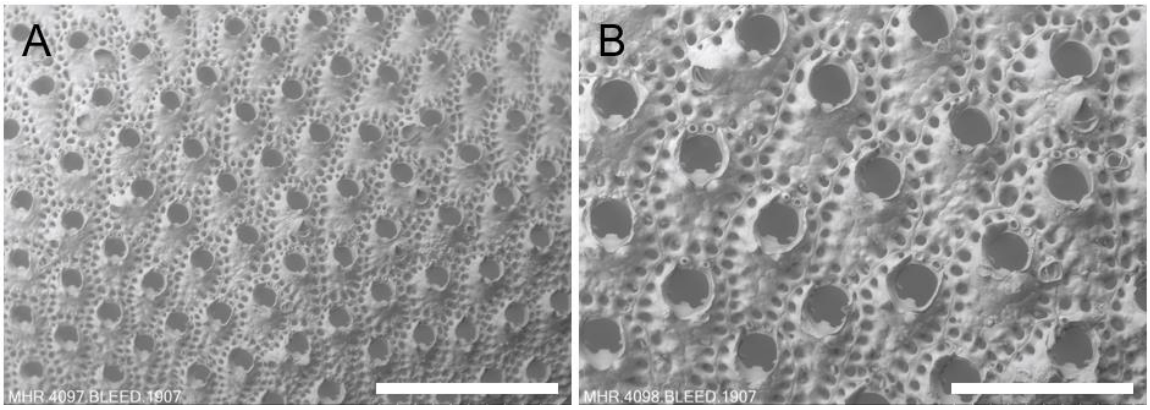
Appendix Figure 14: *Pomocellaria californica*, BLEED 1901, Palmer Point's, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A, B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



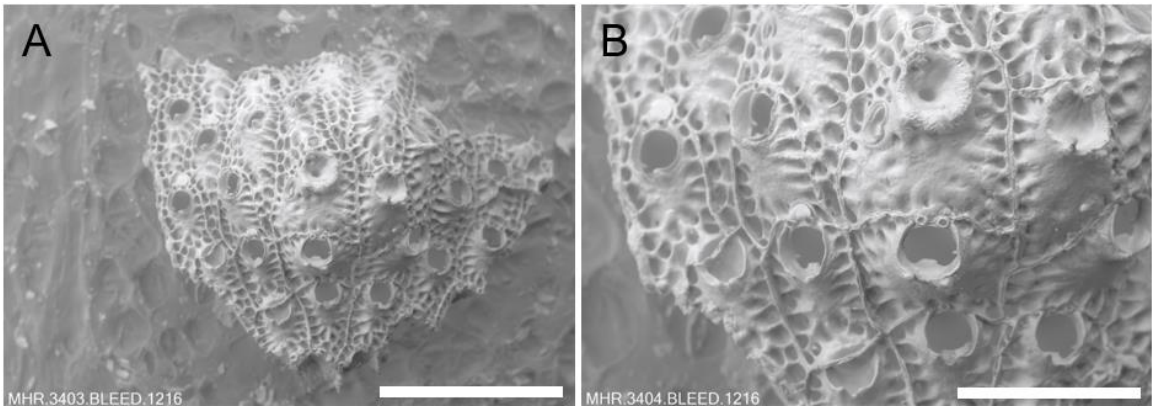
Appendix Figure 15: *Pomocellaria californica,* BLEED 1912, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A, B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



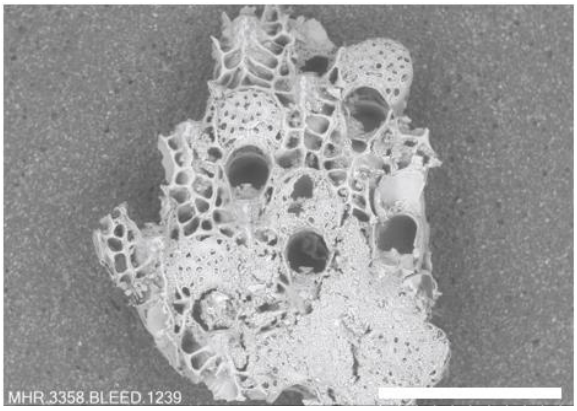
Appendix Figure 16: *Pomocellaria californica*, BLEED 1246, Smithsonian Collection Number 302073, Morro Bay, California, USA. Depth: 1 m. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



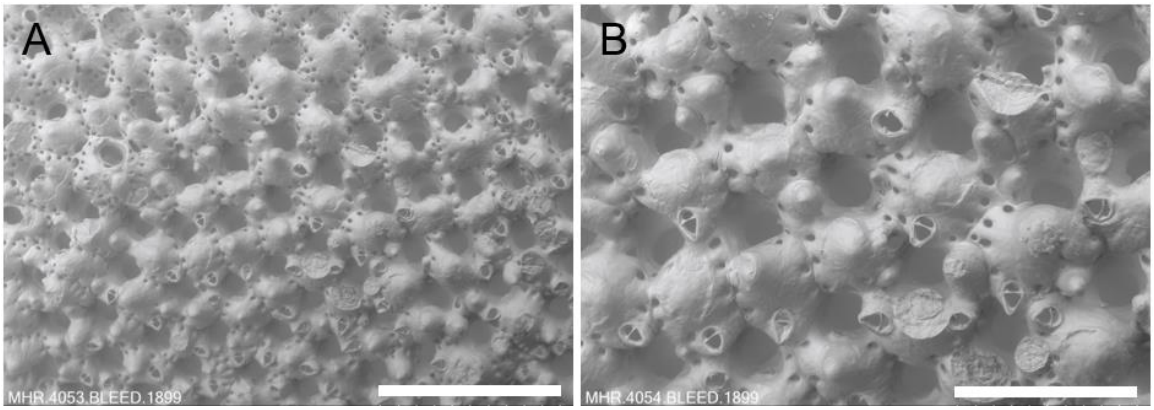
Appendix Figure 17: *Parasmittina collifera,* BLEED 1907, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



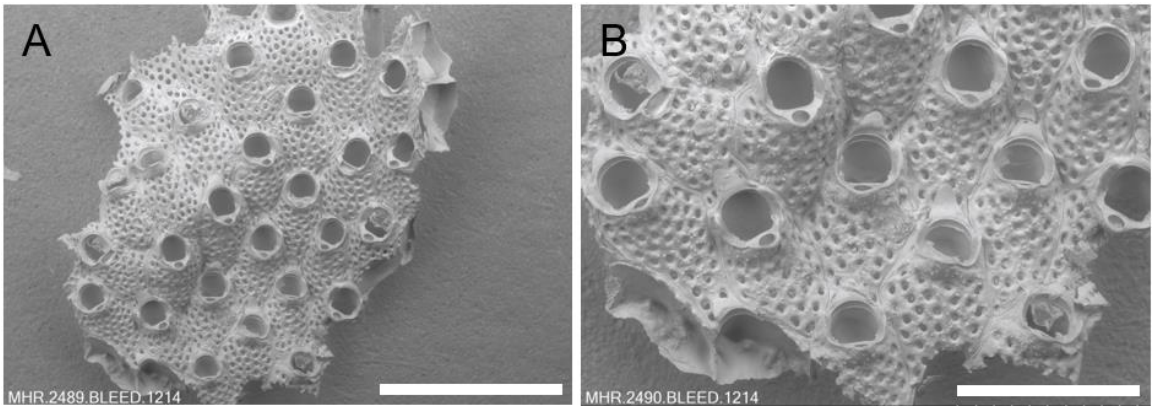
Appendix Figure 18: *Parasmittina collifera,* BLEED 1216; Smithsonian Collection Number 200960, Morro Bay, California, USA. Depth: 1 m. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



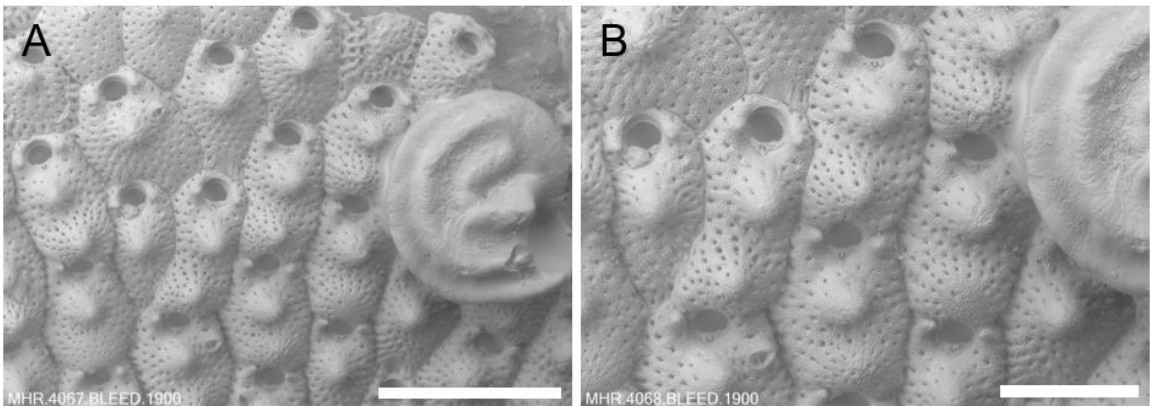
Appendix Figure 19: *Smittoidea prolifica,* BLEED 1239, Smithsonian Collection Number 196262, Loch Lomond Marina, San Francisco, California, USA. Depth: 1 m. Scale Bar: 500 µm. Photo Credit: Mali H. Ramsfjell.



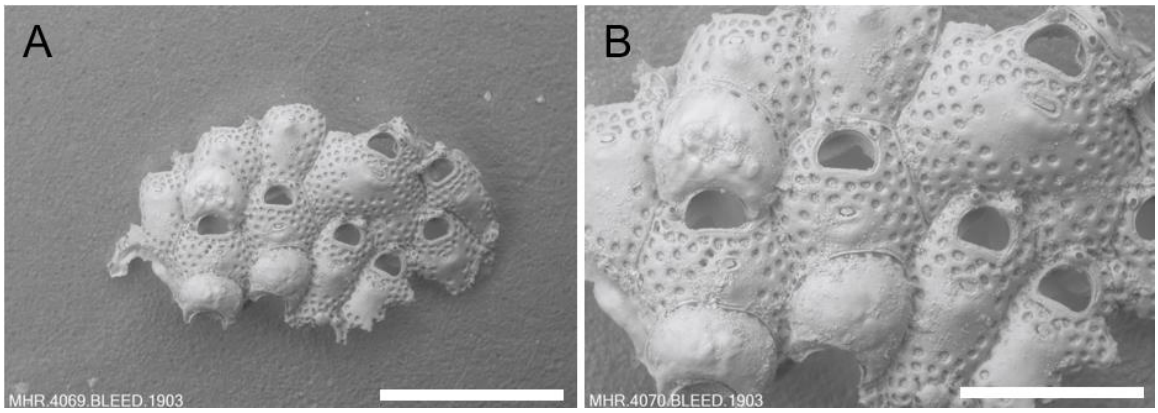
Appendix Figure 20: *Rhynchozoon cf. tumulosum,* BLEED 1899, Palmer's Point, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



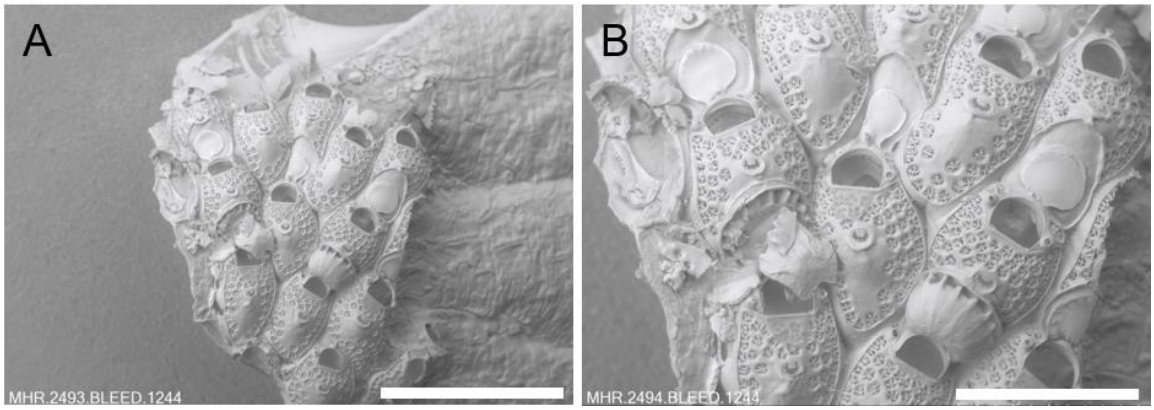
Appendix Figure 21: *Primavelans insculpta,* BLEED 1214, Smithsonian Collection Number 196306, Morro Bay, California, USA. Depth: 1 m. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



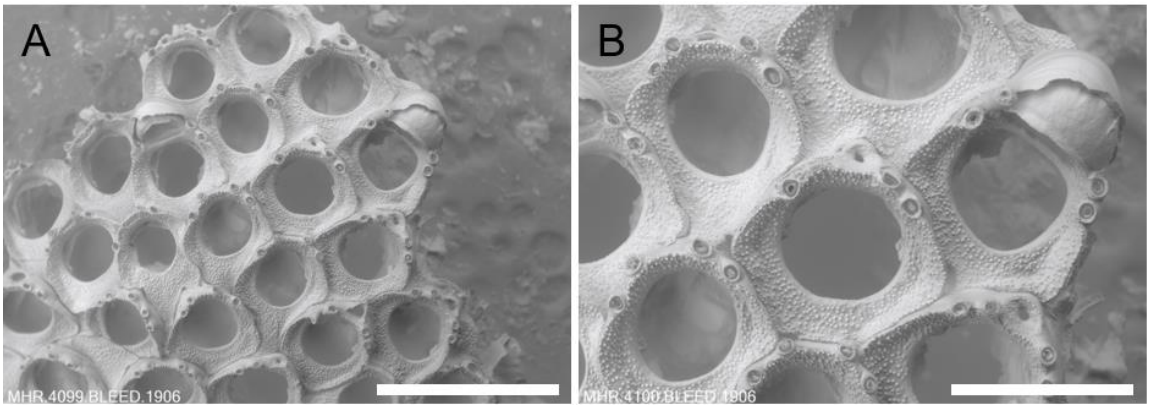
Appendix Figure 22: *Microporella umbonata*, BLEED 1900, Palmer's Point, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



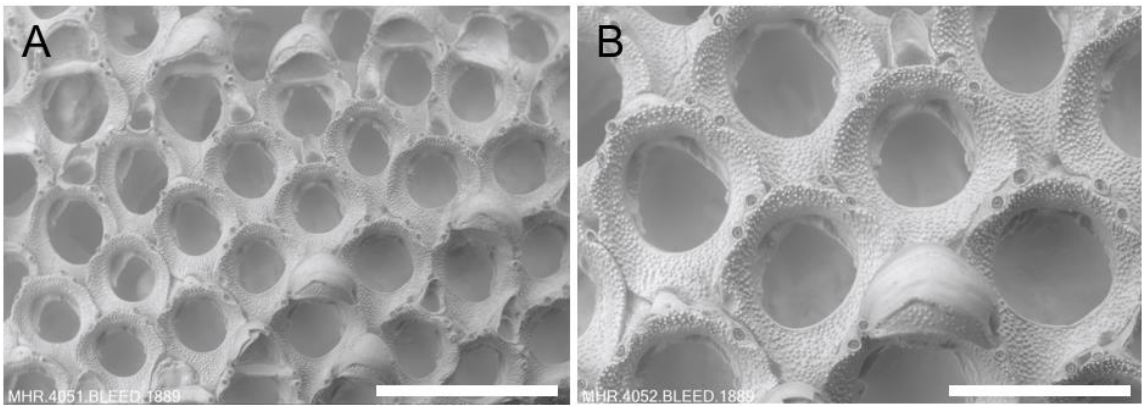
Appendix Figure 23: *Fenestrulina umbonata,* BLEED 1903, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



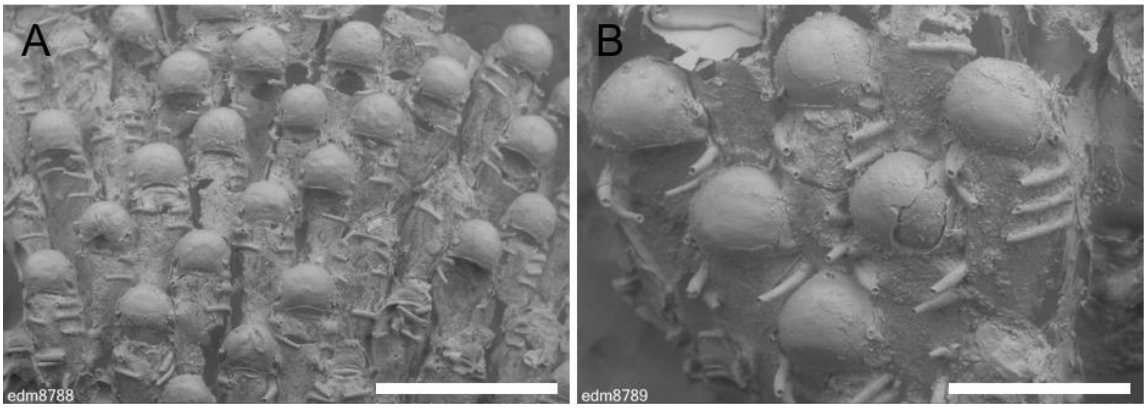
Appendix Figure 24: *Fenestrulina delicia,* BLEED 1244, Smithsonian Collection Number 201938, Morro Bay, California, USA. Depth: 1 m. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



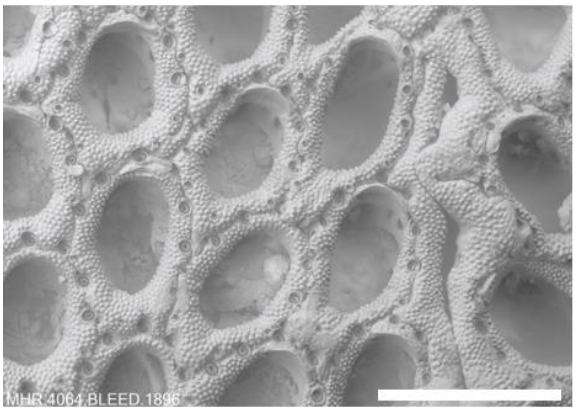
Appendix Figure 25: *Chaperiopsis patula*, BLEED 1906, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



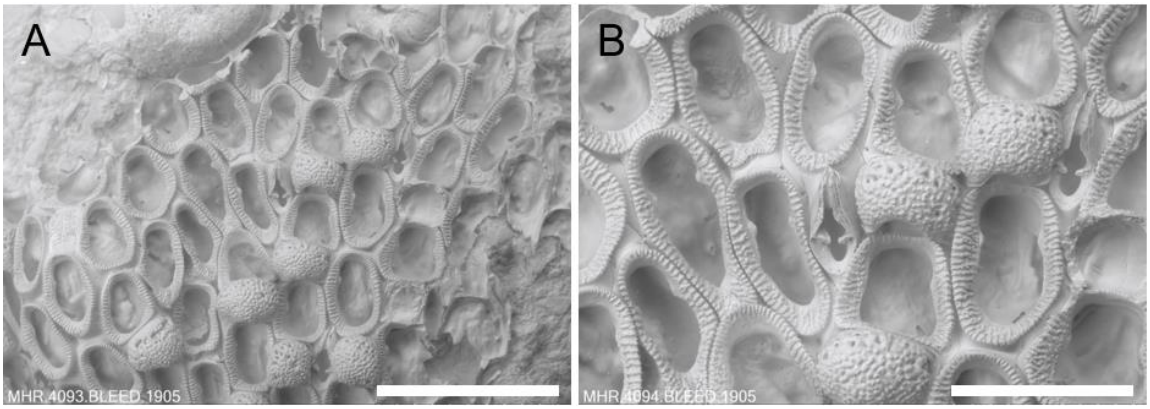
Appendix Figure 26: *Chaperiopsis* cf. *patula*, BLEED 1889, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



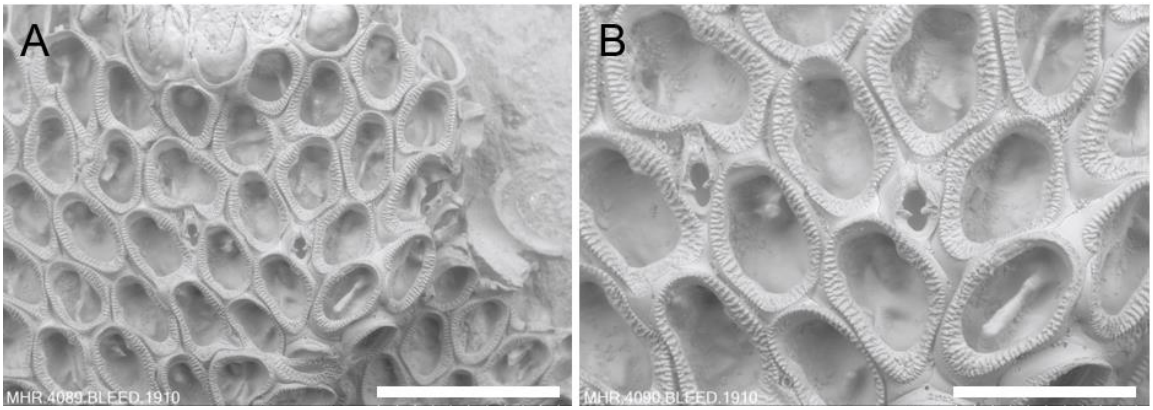
Appendix Figure 27: *Dendrobeania lichenoides,* BLEED 1893, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Emanuela Di Martino.



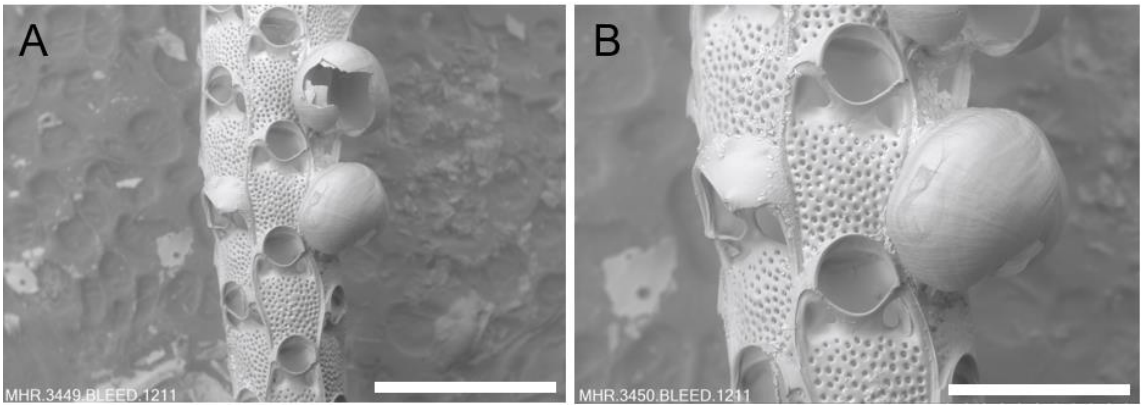
Appendix Figure 28: *Cauloramphus californiensis,* BLEED 1896, Palmer's Point, Trinidad, California, USA. Depth: Intertidal. Scale Bar: 500 µm. Photo Credit: Mali H. Ramsfjell.



Appendix Figure 29: *Copidozoum adamantum*, BLEED 1905, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



Appendix Figure 30: *Copidozoum adamantum*, BLEED 1910, Baker Beach, Trinidad, California, USA. Depth: Intertidal. Scale bar: **(A)** 1 mm; **(B)** 500 µm. Photo Credit: Mali H. Ramsfjell.



Appendix Figure 31: *Thalamoporella californica*, BLEED 1211, Smithsonian Collection Number 248556, Long Beach, California, USA. Depth: 1 m. Scale bar: **(A)** 1 mm; **(B)** 500 μm. Photo Credit: Mali H. Ramsfjell.