Comparative transcriptomics of the two distinct morphologies of the Caribbean octocoral *Briareum asbestinum* (Pallas, 1766)

By:

Liajay Rivera García

A thesis submitted on partial fulfillment of the requirements for the degree of

MASTER OF SCIENCES
in
MARINE SCIENCES
(Biological Oceanography)

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2019

Approved by:

Nikolaos V. Schizas, Ph.D. **President, Graduate Committee**	**Date**
Audrey Majeske, Ph.D. **Member, Graduate Committee**	**Date**
Ernesto F. Weil, Ph.D. **Member, Graduate Committee**	**Date**
Kurt A. Grove, PhD. **Representative of Graduate School**	**Date**
Ernesto Otero, Ph.D. **Director, Department of Marine Sciences**	**Date**

Abstract

Briareum asbestinum (Pallas, 1766) seems to be the only Caribbean species in the genus *Briareum,* with four additional species found in the Indo-pacific region. Since the 1970's, interest from pharmaceutical companies to isolate chemicals from *Briareum* colonies for potential human use led to further investigations of its defense mechanisms and distribution. As in many other octocoral taxa exhibiting diverse morphologies, *B. asbestinum* has been plagued by taxonomic uncertainty. The two common morphotypes, a digitate form and a crustose form (*B. polyanthes*), have been previously reported as separated species. Recent reports however, lump them together into one species. Furthermore, the use of single molecular markers have led to inconclusive results, failing to resolve the ambiguous taxonomic status of the two morphs of *B. asbestinum*. We used new genomic tools (i.e. transcriptomes) to explore and solve the taxonomic status of the two morphotypes found across the southwest coast of Puerto Rico. We used a multi-assembly approach implemented in the NCGAS pipeline. The gene ontology analysis was performed using Trinotate, and genes were categorized by molecular functions, biological processes, and cellular components. The phylogenetic analysis was performed using distinct custom bash programs to select homologous sequences among the transcriptomes, resulting in 2,568 homologous sequences. The concatenation analysis (with traditional Maximum Likelihood) resulted in a topology supporting a clade of *B. asbestinum* shallow species, while the octocoral *Heliopora coerulea* was used as the outgroup. Coalescence-based tree estimation analysis (using RAxML-NG and ASTRAL-II), resulted in a similar topological structure as the concatenated approach. The phylogenetic results showed no significant differences between the two *B. asbestinum* morphotypes, but close genetic similarities, which together with recent

molecular results seem to indicate the presence of a single species in Puerto Rico and the Caribbean.

Resumen

Briareum asbestinum parece ser la única especie caribeña del género *Briareum*, mientras que cuatro especies adicionales se han descrito para la región Indo-Pacífica. Desde los años 1970, el interés de compañías farmacéuticas para aislar químicos de colonias de *Briareum* como potencial para uso medicinal en humanos, ha fomentado investigaciones adicionales de sus, metabolitos secundarios como mecanismos de defensa y su distribución local y geográfica. *B. asbestinum* presenta dos formas de crecimiento: un morfotipo cilíndrico erecto y uno incrustante. Como en muchos otros octocorales con alta variabilidad morfológica, la taxonomía de *B. asbestinum* ha sido confusa. Estos dos morfotipos han sido previamente reportados como especies distintas (*B. asbestinum* y *B. polyanthes*), pero reportes recientes usando marcadores moleculares únicos para cada morfología produjo resultados inconclusos, y otros resultados indican la presencia de una sola especie. En este trabajo usamos nuevas herramientas (transcriptomas) para tratar de descifrar el estatus taxonómico de los dos morfotipos comunes a lo largo de la costa sur-oeste de Puerto Rico y en el Caribe. Usamos un multi-ensamblador implementados en el protocolo de NCGAS. Un análisis ontológico fue hecho a través del programa Trinotate y los genes fueron categorizados como funciones moleculares, procesos biológicos y componentes celulares. El análisis filogenético fue hecho usando distintos programas personalizados en "bash" para seleccionar secuencias homólogas entre los transcriptomas, con un resultado de 2,568 secuencias homólogas. El análisis de concatenación (empleando Máxima Verosimilitud) produjo una homología favoreciendo un clado entre morfotipos de arrecifes someros, utilizando el octocoral *Heliopora coerulea* como grupo externo. Los análisis de incorporación reprodujeron un esquema similar al análisis concatenado. Los resultados filogenéticos indican que no hay diferencias significativas entre los morfotipos de *B.*

asbestinum, pero una una relación genética cercana entre los morfotipos. Estos resultados junto con los últimos análisis moleculares parecen indicar la presencia de una sola especie con alta variabilidad morfológica.

Copyright

Dedication

To Idalia, Jaime and Bolt.

Acknowledgment

I would first like to thank my dear parents for their unconditional support, patience and for always instilling in me perseverance and hard work. To Bolt, my pet, who accompanied me through this journey, bringing happiness and inspiration to work better world, this degree is for you as well.

I would also like to thank my advisor Dr. Nikolaos Schizas for his support, guidance and skills that I have learned through you. Dr. Ernesto Weil and Dr. Audrey Majeske thank you for serving in my committee, and mentoring me. I also wish to thank Martha Ricaurte who was an incredible guide in the laboratory techniques and showed me alternate extraction methods that turned essential to my work. My biggest thanks goes to my brilliant colleague and friend Ramón Rivera Vicéns who helped with the bioinformatics part and whom without his help I would still be learning the basics. To my CIMA friends Mariel, Jaaziel, Duane, Ingrid, Alex, Nick who helped me in the field work, through sampling and document, and with whom we saw many sunsets at the dock. I will forever cherish those sunset group talks. To Luis, my best friend, who helped me most in this thesis from telling species apart, to laboratory techniques and even life skills. May the coffee breaks last forever.

Lastly, I would like to thank Sea Grant Puerto Rico for their financial support to sequence my samples and all CIMA staff, students and faculty for stimulating unity and support within the department.

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1. Introduction

Octocorals of the genus *Briareum* (Blainville 1830) are sparsely distributed on both shallow Caribbean and Indo-West Pacific reefs (Fabricius and Alderslade 2001; Samimi-Namin 2016). Of the five extant species within the genus *Briareum*, only one species, *B. asbestinum* (Pallas 1766) is known to inhabit reefs across the wider Caribbean, Atlantic region, and Bermuda (Bayer 1961; Locke et al. 2013). Commonly known as "Dead man fingers" or "Corky sea finger", *B. asbestinum* is an abundant octocoral distributed along shallow (3 m) to upper mesophotic (40 m) coral reef depths, displaying an adaptation to strong surges, warmer waters and varying light and temperature gradients (West et al 1993). *Briareum asbestinum* displays two main colony morphologies: a digitate erect form and an encrusting-clumped morph, the latter of which is often confused with the octocoral *Erythropodium caribeorum*.

The genus *Briareum* was first described by Pallas (1766), it belongs to the order Alcyonacea (Ehrenberg, 1831), Suborder Scleraxonia, Family Briaridae. Although the *Briareum* genus is one of the most basal genera of Octocorallia (McFadden et al. 2006). Although this genus consists of a few species, the taxonomical status of *Briareum asbestinum* two morphotypes remains controversial. The first taxonomic revision addressing both encrusting and digitate as different species was proposed by Duchassaing and Michelotti (1860) and the crustose form was named *B. polyanthes*. Since the original description, *B. asbestinum* morphotypes have undergone different taxonomic assignments and its morphological plasticity has hindered the taxonomic understanding of this genus (Miyazaki 2014). Bayer (1961) synonymized both growth forms as a single species *B. asbestinum*. Harvell et al. (1993) performed a short-term transplant suggesting a genotypic difference from both morphotypes. However, this was followed by the work of Brazeau and Harvell (1994), which used allozyme data suggesting no differences between the

two morphs. Sánchez and Wirshing (2005) provided a field key guide based on morphological characters, ranging from colony branching patterns, surface morphology and texture, extended polyp size, and colony color, separating both forms and describing the encrusting morphotype as *Briareum polyanthes*. Later on, Bilewitch et al. (2010) re-examined genetic and morphological characteristics of the encrusting morphology from Bermuda and of both morphotypes from the Bahamas, and concluded that their nuclear ribosomal data contained multiple unique genotypes within and among individuals, but the high intragenomic variation required extensive screening to determine the homology of haplotypes, rendering their results inconclusive. Despite the high quality of all the previous works, the molecular characterization of the digitate and encrusting morphotypes of *B. asbestinum* remains ambiguous by virtue of the currently limited availability of molecular markers. To this day, both morphs have been inconsistently referenced by marine chemistry and ecology researchers as well as taxonomists (Grode et al. 1983; Rodríguez et al. 1996; Berrue and Kerr 2009; McClanahan et al. 2018).

During the past three decades the families *Briaridae, Gorgoniidae and Plexauridae* have received major attention from pharmacological companies because they provide natural secondary compounds, from which novel products can be created with potential use in pharmaceutical applications (Berrue and Kerr 2009). One of these natural compounds are diterpenes, which contain several biological activity properties such as anti-inflammatory and fish deterrence (Burks et al. 1977; Lei 2016). To date, there are more than 30 scientific publications based on isolation of secondary metabolites from *B. asbestinum* (i.e. Grode et al. 1983; Rodríguez et al. 1996; Lei 2016).

It is important to recognize that cause of morphological plasticity and decipher whether is adaptive and therefore increasing survivorship and fitness of species. In many benthic marine

organisms, morphology changes as a response to different environmental and biological pressures (i.e. water energy, quality and quantity of light, substrate profile, predation or competition pressures, etc.) (West et al. 1993, 1997; Hammerschlag 2018; Rambsy 2019). The different morphologies of *B. asbestinum* may represent adaptations to exposure to shallow-reefs with high energy wave motion and strong currents (West 1997). A transplantation and fragmentation study by West (1997), showed a great capacity for *B. asbestinum* to change sclerite length according to depth and light exposure.

With the advent of next generation DNA sequencing technologies we can revisit such questions with enormous amounts of data compared to previous research. Phylogenetics has greatly benefited by the advanced sequencing technologies as 100s to 1000s orthologous genes can be obtained through sequencing of transcriptomes or genomes. Transcriptome-based sequence data sets are relatively inexpensive to produce and have produced new questions and phylogenetic insights (Oakley et al. 2012; Washburn 2017). Notable phylotranscriptomic work by Kocot et al. (2011) and Weigert et al. (2014) reconstructed the phylogenetic relationships of molluscans and annelids, respectively. More recently, Zapata et al. (2015) used a combination of genomic and transcriptomic information (a total of 1,262 genes) of different groups of Cnidaria to decipher their phylogenetic relationships. These results contributed to Kayal et al. (2018) clarification on the cnidarian tree of life, where they proposed a new hypothesis for cnidarian phylogeny based on new and existing genome-scale data that included representatives of all cnidarian classes.

The goal of this project is to contribute to the taxonomic relationship of the digitate and encrusting morphotypes of *Briareum asbestinum*. This was accomplished by generating phylogenetic trees from data obtained through transcriptome sequencing. A second goal is to

report the first transcriptomes of the two morphotypes of *B. asbestinum*. Since samples were collected from two depths, additional transcriptome analysis was performed to assess spatial variability across depth habitats.

2. Literature Review

2.1 Classification

Domain: Eukaryota

Group: Opisthokonta

Kingdom: Metazoa

Subkingdom: Eumetazoa

Phylum: Cnidaria

Class: Anthozoa (Ehrenberg 1831)

Subclass: Octocorallia (Haeckel 1866)

Order: Alcyonacea (Lamouroux 1812)

Sub Order: Scleraxonia (Studer 1887)

Family: Briareidae (Blainville 1830)

Genus: *Briareum* (Blainville 1830)

Species: *Briareum asbestinum* (Pallas 1766)

2.2 Genus description and distribution

The genus *Briareum* consists of five species, where *B. asbestinum* is endemic and the only species in the Caribbean. The other four species are distributed through the Indo-Pacific region. Common traits include an encrusting colony shape, a cortex with magenta or colorless sclerites (Fabricius and Alderslade 2001), and a medulla containing magenta-colored sclerites. These morphological characters in *Briareum* species can show high variation in response to

environmental factors such as depth, water motion, light, and predator damage (West 1997). The following subsections describe a brief key characteristic for each species in the Indo-Pacific region, followed by single species *B. asbestinum* in the Caribbean region.

2.2.1 Indo-Pacific (**Appendix, Figure 10A)**

B. hamrum (Gohar 1948) (**Appendix, Figure 10A: A-B**): Its coenenchymal spindles are up to 0.45mm long with prominent, sparsely set tubercles. A recent overview of Indo-Pacific species by Samimi-Namin and van Ofwegen (2016) reports that *Briareum hamrum* is exclusively found in the northwestern Indian Ocean, Seychelles, and East Africa coast. Its distribution includes the Red Sea, Oman Sea, Arabian Sea, and Persian Gulf.

B. stechei (Kükenthal 1908) (**Appendix, Figure 10A: C-D**): Contains numerous spindles with blunt ends in its coenenchyme, sclerites are magenta and colorless (Stiasny 1937). It has been reported in Coral Triangle, Australia (Low Isles), Guam, and Taiwan.

B. violaceum (Quoy & Gaimard 1833) (**Appendix, Figure 10A: E-F**): Have many spindles with pointed ends in coenenchyme, all sclerites are magenta colored. It has been found in Vanuatu, Japan (Ryukyu Archipelago, Bonin Islands), Taiwan, Coral Triangle, Australia (Great Barrier Reef).

B. cylindrum (Samimi-Namin and van Ofwegen 2016) (**Appendix, Figure 10A:G**): Have many cylinders present in coenenchyme, with dense tuberculation. It resembles *B. cylindrum* but is distinguished by the many cylinders with complex tubercles in the coenenchyme. It is distributed along Australia, Coral Triangle and China.

2.2.2 Caribbean and Atlantic Ocean

B. asbestinum (Pallas 1766): Of the five extant species, *B. asbestinum* **(Figure 1)** is the only species of the genus inhabiting the Caribbean and southern Atlantic region with

noticeable presence at higher latitudes in Bermuda (Bayer 1961; Locke et al. 2013; Velásquez and Sánchez 2015).

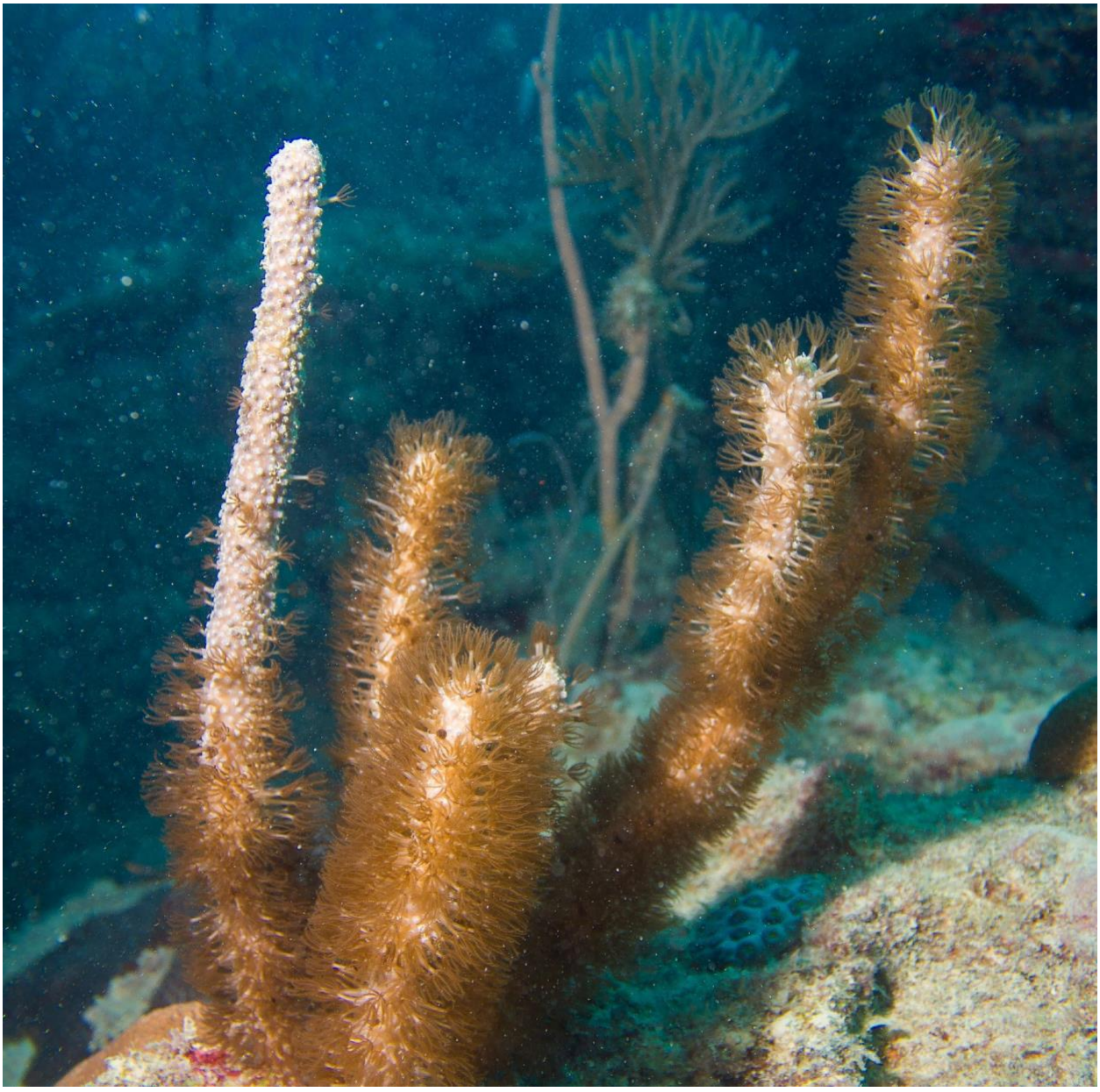

Figure 1A) Digitate and **1B)** encrusted forms of *B. asbestinum.*

2.3 *B. asbestinum* species description

First described by Pallas (1766), *B. asbestinum* exhibits two distinct colony forms in the Caribbean: a digitate form **(Figure 1A)**, and an encrusted form **(Figure 1B)** to which the common name "Corky sea finger" or *"Deadman's finger"* is ascribed to frequently by aquarists. The digitate form can appear sharing a stolonal mat with other branches, while the encrusting form appears as clumps or a flat tissue over the substrate. The crustose form overgrows hard substrates on once living corals of different growth forms, including other erected coral species, sometimes, mistaken as its digitate form. Reports show a growth rate of 2.02 cm/year for individual branch measurement of the digitate morphology (Brazeau & Lasker 1992); an average growth among shallow water octocorals of the Caribbean (Yoshioka & Yoshioka 1991) and significantly lower than scleractinians with branching growth such as *Acropora cervicornis*

(Gladfelter et al. 1978). Its coenenchyme can be brown or purple in color with extended brown polyps. However, darker purple spots show over the retracted polyp opening; a main feature that helps to discern from the paler looking polyps from *Erythropodium caribeorum* **(Figure 2)**.

B. asbestinum polyps have an average diameter between 17mm-18mm, lack sclerites, and possess eight pinnated tentacles in each polyp that when expanded present a brown color (Gray 1859). Bilewitch et al. (2010) reported a mean length of sclerites of approximately 300 μm for both morphotypes and suggested that although variations can be found within a growth form, sclerite length alone cannot be taken as evidence of genetic divergence. As all soft corals in the order Alcyonacea, *B. asbestinum* sclerites act as rigid reinforcing points of attachment within the soft matrix, providing resistance to deformation; smaller sclerites length along with greater densities provide greater support to erect protrusions and flat surfaces. (Koehl 1982; Palumbi 1986).

Figure 2. *Briareum asbestinum* (left) with purple polyp opening and *Erythropodium caribeorum* (right) with pale beige polyp opening.

2.4 Feeding and nutrition

Most corals species associate with endosymbiont algae commonly called zooxanthellae that transfer part of their photosynthate to their host (Muscatine and Cernichiari 1969) resulting in a contribution to the metabolic needs of these corals. The majority of Caribbean octocorals

harbor the zooxanthellae *Symbiodinium* clade B (Ramsby et al. 2014). Recent studies provided by Hannes et al. (2009) and Ramsby & Goulet (2019) identified *Symbiodinium* B1 or B19 on *B. asbestinum* encrusted colonies and B1, B19, B21 or B33 on digitate morphologies. Experimental work by Poland et al. (2013) demonstrated that different symbionts continued to enter the host from the water column for months until only one kind dominated within the juvenile *B. asbestinum*. Still yet, additional feeding mechanisms from the coral itself are employed to meet the energy demands for its proper functionality.

The feeding strategy of *B. asbestinum* consists of prey capture by its polyps. As the polyp extend its long tentacles into the water column, food particles become entrapped in their pinnules, followed by a synchronized, inward contraction (Lewis 1982). Upon small particulate food capture the tentacle will rapidly fold inwards toward the mouth so the particle can be suctioned into the pharynx. Larger food entrapment requires a coordinated inward movement of all eight tentacles writhing over the oral disk. Laboratory observations by Lewis (1982) report *B. asbestinum*, as other octocorals, feeding on ground fish, brine shrimp and live zooplankton.

2.5 Reproduction

B. asbestinum is a gonochoric octocoral, capable of sexual and asexual reproduction. Sexual reproduction occurs after full moon of June and July where male colonies release their sperm into the water column and are transported through current and proximity to the female polyp so internal fertilization takes place (Brazeau 1989; Brazeau and Lasker 1990). Once fertilized, embryonic development occurs in the gastro-coelenteron, and then, the early-stage embryos are brooded from female polyps but, remain attached (within the mucus layer) to the colony from 3 to 5 days while completing embryogenesis and developing into aposymbiotic planulae. Mature planulae will then be synchronously detached from the parent colony into the

water column. Since they are negatively buoyant and competent for attachment, they will settle shortly after and start asexual reproduction of new polyps so the colony can grow fast (Kinzie 1974). Settlement occurs mostly on hard substrate as it provides more stability for the octocoral (Kinzie 1973). Other types of reproduction include fragmentation of branches and fission of crustose tissues that produce ramets (clones) that re-attach to the substrate and grow into separate, but genetically identical colonies (Lasker 1983).

2.6 Stress and mortality: Defense against predation and bleaching events

B. asbestinum lack stinging nematocysts, nonetheless, Sebens and Miles (1988) described them capable of producing sweeper tentacles capable killing tissues of space competitors. Octocorals in general produce high amount of secondary compounds that may function as predator and competitor deterrents (Harvell and Fenical 1984). The first report of secondary compounds on *B. asbestinum* was published by Burks et al. (1977). They isolated a type of diterpene eventually named "Briarane A". This class of diterpenes has been reported to contain a wide range of cytotoxicity toward tumor cell lines (Berrue and Kerr 2009) as well to suppress the expression of pro-inflammatory proteins involved in the progression of Parkinson's disease (Chen et al. 2012). Since then, *B. asbestinum* and many other octocorals have been studied for their production of secondary metabolites that have been shown to have important biological functions (cytotoxicity, antimicrobial, anti-inflammatory, antiviral, immunomodulatory, antifouling, and ichthiotoxicity) (Lei 2016). They can be isolated and today, have been synthetically replicated for pharmaceutical and medical use. Although some compounds together with spicules (West 1997, 98) serve as deterrence for most predators, *B. asbestinum* could suffer high mortalities by the snail predator *Cyphoma gibbosum*, the fireworm *Hermodice carunculata*, and the fish *Chaetodon capistratus* (Harvell et al. 1996; West 1998; Lucas et al. 2014).

High water temperatures are responsible for the ever-increasing incidents of massive coral reef bleaching over the last three decades (Harvell et al. 2001; Donner et al. 2005; Prada et al. 2010; McClanahan et al. 2018). Bleaching events compromise coral immunity increasing the probability for subsequent effects such as disease, reduced growth, reproduction and death that have accelerated coral reef degradation world-wide. Although *B. asbestinum* has been susceptible to bleaching events and diseases (Kim et al. 2017; Weil et al 2018), it exhibits high resistance and resilience to environmental perturbations, particularly to thermal stress, compared to scleractinians (Guzman et al. 2018). Moreover, *B. asbestinum* can shift symbionts as was shown after an artificial bleaching event (Lewis and Coffroth 2004; Hannes et al. 2009).

Diseases are considered as another mortality contributor of *B. asbestinum*. An infected digitate morphotype will experience coenenchyme tissue loss, consequently exposing their spicular axis. Harvell et al. (2001) reported an epizootic event coincident with a bleaching event in 1998; suggesting that the disease as an opportunist infection. Moreover *B. asbestinum* have been impacted by diseases such as aspergillosis in the Caribbean (Weil and Rogers 2011). Although a cyanobacteria *Scytonema* sp. was tentatively identified, more studies are needed to determine a definitive pathogen.

3. Objectives

1. To generate independent *de novo* assembly of the transcriptome of both morphologies of the octocoral *Briareum asbestinum* using RNA-seq, a NGS application.
2. To compare the intergenomic variability in the transcriptome of both morphotypes within the same and across different habitats (shallow vs. deep)

3. To perform a phylogenetic analysis to discern the taxonomic status of the two morphotypes in *B. asbestinum*.

3.1 Question and Hypothesis

1. Do the two morphotypes of *B. asbestinum* represent different species, or divergent genetic lineages within a species? Are the two morphotypes of *B. asbestinum* different at the transcriptome level within each habitat and across habitats?

$H0_1$: There are no differences between the transcriptomes of the crustose and the digitate forms in *B. asbestinum*- (they are the same species.)

$H1_1$: There are significant differences between the transcriptomes of the crustose and digitate forms in *B. asbestinum*- (they are different species).

H_{02}: There are no spatial (shallow vs. deep) differences in the transcriptomes of each morphology and between morphologies

H_{12}: There are significant spatial (shallow vs. deep) differences between the transcriptomes of each morphology and between morphologies.

4. Materials and Methods

4.1 Study Sites and Field collection

Colonies of both the digitate and encrusting morphotypes were sampled at 5 m depth in Turrumote reef (n=4) and at 20 m depth at the continental shelf edge (n=4) in the La Parguera Natural Reserve, Puerto Rico (**Figures 3 and 4, Appendix Table 1**) on February 2017. These reefs were selected for specimen abundance, and proximity to Isla Magueyes Laboratories of the Department of Marine Sciences, University of Puerto Rico, Mayagüez. Open circuit SCUBA diving was used to collect the samples and tissue was separated from colonies using a knife by

cutting about 10 cm^2 of tissue approximately 10 cm from the tip of a branch and from the centermost area of a crustose colony. Samples were collected from colonies that were more than 10 m apart to reduce the possibility of collecting from clone-mates (Lasker 1983). Samples were collected under a research permit from the Department of Natural and Environmental Resources of Puerto Rico (O-VS-PVS15-MA- 00016-26,092,014). The tissue samples were placed in sterile, plastic bags and once on the boat, placed immediately on ice and transported to the lab, where they were rinsed and placed in a labeled, dry zip-lock bag in stored at -80ºC until further processing.

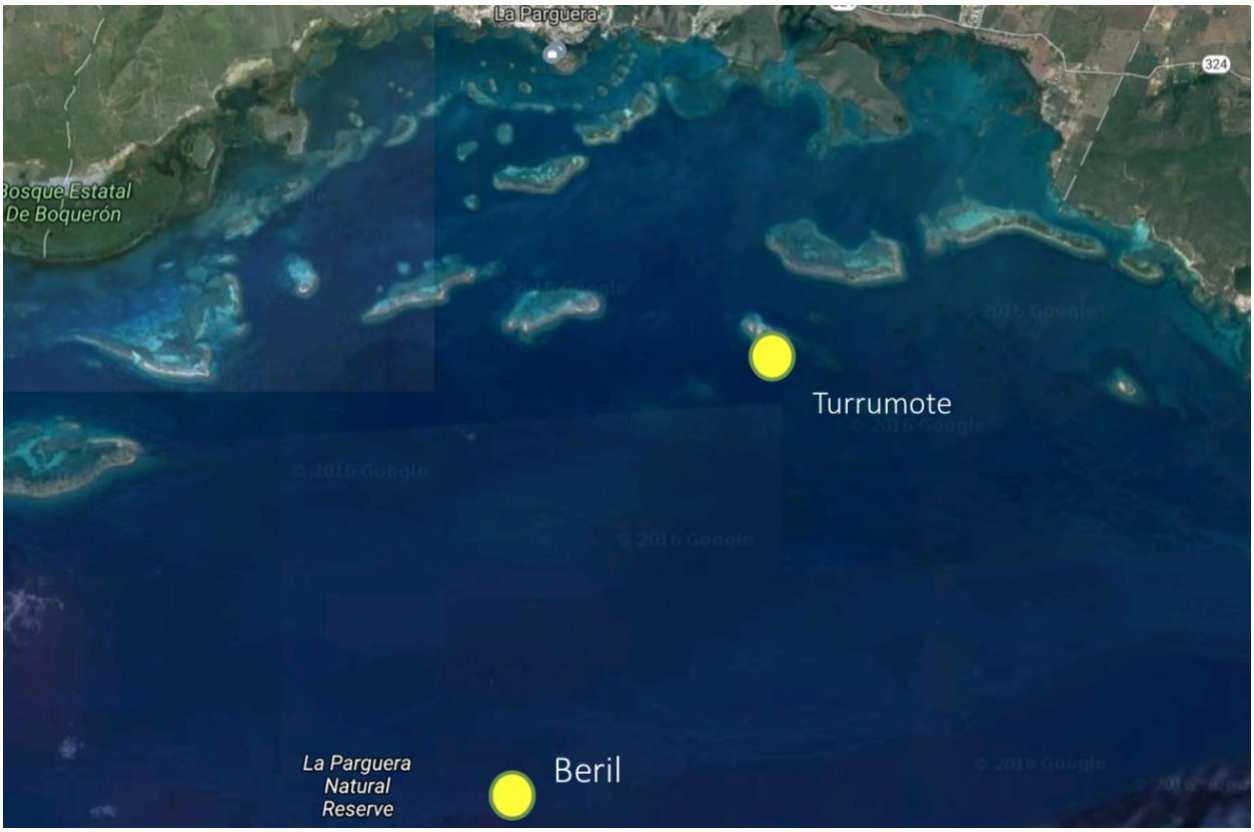

Figure 3: Sampling site of shallow and deep samples, Turrumote II and Beril reefs .

Figure 4: Collection method.

4.2 Molecular techniques and sequencing

Tissue was triturated through short intervals of sonication and total RNA was extracted using the TRIZOL RNA Isolation method (Chomczynski and Mackey 1995). Extractions were done thrice per sample (i.e. three pseudo-replicates RNA extractions from each colony) followed by pooling all three to obtain high yield of RNA. To assure we obtained a high yield of RNA from all samples we measured their RNA quantity and quality using NanoDrop 2000 (Thermo Fisher Scientific) prior being sent for sequencing. A total of 16 samples (eight from shallow reef habitats and eight from deep reef habitats) were sent for further processing to Duke Center for Genome and Computational Biology in Duke University. Prior to sequencing, further quality and quantity assessment (2100 Agilent Bioanalyzer) was done and sequencing libraries (n=8) were prepared using the KAPA Stranded mRNA Seq Kit (Cat # KK8421). Total RNA sequencing was performed on all samples with poly-A tail selection on an Illumina NextSeq 500 platform with 150 bp paired-end reads.

4.3 Transcriptome assembly

4.3.1 *De novo* transcriptome

The first high quality *de novo* transcriptome of the digitate morphotype of *B. asbestinum* was published (Rivera et al. 2019) following the assembly strategy described in Veglia et al.

(2018), which incorporates comprehensive contamination removal prior to *de novo* assembly. Results from Veglia et al. (2018) showed that pre-assembly contamination removal in combination with a multi-assembler approach greatly improved *de novo* coral transcriptome assemblies. This protocol was also employed on the transcriptome assembly of the encrusting morphotype.

Sequence libraries produced were then verified for their quality by using the program FastQC v0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). This program assesses the reads' quality and determine any possible contamination by sequencing adapters and barcode presence. Adapter sequences were removed from libraries using FastX-ToolKit (http://hannonlab.cshl.edu/fastx_toolkit/index.html) to generate higher quality reads. All reads with an average per base Phred score below 30 were removed before assembly. Reads libraries were scanned for possible biological contamination using NCBI BLAST v2.7.1 (Altschul et al. 1990) and three contamination databases: *i.* Custom *Symbiodinium* genome database [Shoguchi et al. (2013; *S. minutum*); Lin et al. (2015; *S. kawagutii*); Aranda et al. (2016; *S. microadriaticum*); Liu et al. (2018; *Symbiodinium* clade C1); and unpublished data from the ReFuGe 2020 Consortium, C.X. Chan, P. Lundgren, C.R. Voolstra (*Symbiodinium* clade C15)] , *ii.* Marine bacteria database [MarDB V2 (https://mmp.sfb.uit.no/databases)] and the *iii.* NCBI contamination database for eukaryotic sequences. All reads with a hit to any of the three custom databases were removed from the read files prior to assembly and stored for downstream taxonomic analysis. Read libraries were then quality checked again with FastQC v0.11.5 before proceeding to the *de novo* assembly to verify its quality content change.

High quality reads were then assembled utilizing the multi-assembler assembly pipeline developed by the National Center for Genome Analysis Support (NCGAS). The NCGAS

pipeline is initiated by generating multiple *de novo* assemblies using varying kmer lengths (e.g. 35, 45, 55, 65.) and assembly programs [Trinity v2.6.6 136 (Grabherr et al. 2011), SOAPdenovo-Trans (Xie et al.2014), Velvet v1.2.10 (Zerbino et al. 2008), Oases v0.2.09 (Schulz et al. 2012), Trans-ABySS (Robertson et al. 2010)]. EvidentialGene v2013.07.27 (Gilbert 2012), a genome informatics program, was then used to generate a high quality consensus *Briareum asbestinum* transcriptome assembly.

We then employed QUAST v4.6.3 program (Mikheenko et al. 2016) to assess the basic statistical analyses once our high quality consensus transcriptome was assembled. The Transcriptome Shotgun Assembly (TSA) check steps from NCBI were used to confirm that the transcriptome of *B. asbestinum* was contamination-free prior to further analysis. For transcriptome completeness assessment we used BUSCO v3.0.2 (Simão et al. 2015; Waterhouse et al. 2017) with the metazoans single-copy orthologs reference database.

4.4 Sequence functional annotation

Filtered Open reading frames (ORFs) from the transcripts allows further transcriptome and statistical analysis by blast searches, annotation and gene ontology. To identify and extraction of ORFs we ran a script on EvidentialGene; using as reference the UniProtKb/Swiss-Prot and the PFAM database (2018-04; Finn et al. 2016). Trinotate was used to generate blast searches, functional annotation and gene ontology assignment of identifies ORFs.

4.5 Expressed genes analysis and comparisons with other Octocorals

Guzman et al. (2018) recently published the genome of the octocoral *Heliopora coerulea*, filling a phylogenetic gap within the cnidarians. *H. coerulea* is an octocoral located in the Indo-Western Pacific region, Red Sea, American Samoa, southern Japan and Australia. Both *H. coerulea* and *B. asbestinum* belong to the same subclass Octocorallia (*H. coerulea*, Order:

Helioporacea; *Briareum*, Order: Alcyonacea), this enables a meaningful comparison against the ingroup *Briareum* as an appropriate outgroup for phylogenetic and gene expression comparison.

4.6 Phylogenetic analysis

The filtered ORFs were used as input for an All-versus-All-Blast search of the proteins. A custom bash script was created to search the sequences that had >70% similarity, an E-value lower of $1e^{-5}$ and meet the requirement of the reciprocal match. The sequences that met these criteria were clustered in separate files, with a representative of each species of *Briareum* and depth, using a script. All *Heliopora coerulea* protein sequences, available at the moment in UniProt, were downloaded for further comparison with the *Briareum* proteins. A bash program was used to find the *Heliopora coerulea* sequences that had more than 25% of similarity with the *Briareum* sequences via Blast. Results of these searches were put it in the same file with the other four *Briareum* sequences. These sequences were then used for the phylogeny reconstruction.

4.6.1 Concatenation of DNA sequences

All sequences were concatenated in order to avoid phylogenetic noise from big data sets such as the one presented here. This fundamental way of phylogenetic reconstruction is useful in cases where noise presence is a primary source of conflict and gene-tree methods for reconstruction are useful when conflicted phylogenetic signal is attributed to incomplete lineage sorting (ILS) (Ortiz 2018). For the concatenation analysis, we aligned each file separately using the alignment program MAFFT (Katoh and Standley 2013). Each alignment was then concatenated into a separate file per species using a custom bash script. After each file was created, we then concatenated all species in one file for performing the super alignment using MAFFT in CIPRES (Miller et al. 2010). We used the program ProtTest (Abascal et al. 2005) to

select the best model for amino acid substitution for phylogenetic analysis. The program was first run using all the *B. asbestinum* proteins with *Heliopora coerulea* as outgroup and then run a second time without *H. coerulea*. The substitution models obtained were then applied to each program, if available. We performed Maximum Likelihood analysis using the program RAxML-NG (Stamatakis 2014; Kozlov et al. 2018) with the model GRT+GAMMA (Le and Gascuel 2008) for the dataset with *H. coerulea* as the outgroup. The program was run for each dataset using the rapid bootstrapping method search option (-f a) with 1,000 bootstraps. All trees were visualized using the FigTree v1.4.3 program (http://tree.bio.ed.ac.uk/software/figtree/).

4.6.2 Coalescence

For the coalescence analysis, we used only the dataset with *Briareum* sequences. We ran RAxML-NG for every single gene file using the model GTR+GAMMA and the rapid bootstrapping method (-f a) with 1,000 bootstraps. All the gene trees generated along with their bootstrap replications were used as input for the program Astral-II (Mirarab and Warnow 2015). Astral was run with the full annotation mode (-t 4) and 100 replicates. All other parameters were left on default. All trees were visualized using FigTree.

5. Results

5.1 Sequence Data

The sequences were checked for quality and read content. Sequences from the Deep digitate colonies yielded the highest quantity of paired reads (83,243,170), while sequences from deep reefs yielded the highest quantity among the combined sequences (**Table 1, Table 2**). Meanwhile, the digitate shallow sequences yielded the lowest number of paired reads (76,511,532) (**Table 1**).

5.2 Assembly

Of all the assembled transcriptomes after EvidentialGene, the deep digitate transcriptome yielded the biggest assembly with 404,660 contigs and an N50 of 1,407.

5.3 Transcriptome quality assessment

The quality and completeness of the transcriptomes were assessed with BUSCO. We searched for the Metazoans orthologs sequences that were present in our assemblies and compared them to the total amount of orthologs shared by metazoans. All the *Briareum* transcriptomes had similar numbers of complete orthologs but differed on missing, fragmented and duplicated orthologs (**Table 1, Table 2**). The digitate shallow transcriptome had the most complete orthologs, and the least missing/fragmented orthologs (**Table 2**).

5.4 Normalization

After filtration was done with EvidentialGene, we obtained the highest number of sequences from the digitate form with fewest sequences from the encrusting form.

Sequences and filtering				
Sequencing reads (R1, R2)	Number of Sequences	Median length		
Digitate (shallow + deep)	159,754,702	150		
Encrusting (shallow +deep)	157,548,862	150		
Deep combined (encrusted + digitate)	161,410,462	150		
Shallow combined (encrusting + digitate)	155,893,102	150		
Total Assembly				
Contigs (k>200)	Contigs (before Evigenes)	Contigs (after Evigenes)	N50 (bp)	
Digitate (shallow + deep)	23,371,482	371,554	1,500	

Encrusting (shallow + deep)	20,104,551	416,410	1,441	
Deep combined (encrusted + digitate)	21,006,525	411,980	1,466	
Shallow combined (encrusted + digitate)	20,814,437	405,806	1,480	
BUSCO				
Total BUSCO: 978	Complete	Complete and duplicate	Fragmented	Missing
Digitate (shallow + deep)	943	66	10	25
Encrusting (shallow + deep)	947	187	21	10
Deep combined (encrusted + digitate)	940	193	18	20
Shallow combined (encrusted + digitate)	943	68	17	18
Trinotate: Gene ontologies				
	Total transcripts			
Digitate (shallow + deep)	23,744			
Encrusting (shallow + deep)	29,307			
Deep combined (encrusted + digitate)	27,383			
Shallow combined (encrusting + digitate)	25,743			
TransDecoder				
	Total number of ORFs	Complete ORFs		
Digitate (shallow + deep)	102,839	34,067		
Encrusting (shallow + deep)	120,761	37,916		
Deep combined (encrusted + digitate)	116,239	40,237		

Shallow combined (encrusted + digitate)	110,558	35,968		

Table 1: Transcriptome information, data, assembly, quality, filtration and ORFs of combined samples per morphotype and per depth.

Sequences and filtering				
Sequencing reads (R1, R2)	Number of Sequences	Median length		
Digitate shallow	76,511,532	150		
Digitate deep	83,243,170	150		
Encrusting shallow	79,381,570	150		
Encrusting deep	78,167,292	150		
Total Assembly				
Contigs (k>200)	Contigs (before Evigenes)	Contigs (after Evigenes)	N50 (bp)	
Digitate shallow	11,737,257	262,892	1,508	
Digitate deep	11,634,225	404,660	1,407	
Encrusting shallow	13,720,271	305,143	1,359	
Encrusting deep	11,136,912	387,293	1,289	
BUSCO				
Total BUSCO: 978	Complete	Complete and duplicate	Fragmented	Missing
Digitate shallow	947	53	7	24
Digitate deep	927	178	22	29
Encrusting shallow	922	79	32	24
Encrusting deep	916	190	19	42
Trinotate: Gene ontologies				

	Total transcripts			
Digitate shallow	22,310			
Digitate deep	27,383			
Encrusting shallow	24,127			
Encrusting deep	25,824			
TransDecoder				
	Total number of ORFs	Complete ORFs		
Digitate shallow	88,494	28,481		
Digitate deep	81,019	25,076		
Encrusting shallow	98353	26208		
Encrusting deep	78,769	24,876		

Table 2: Transcriptome information, data, assembly, quality, filtration and ORFs of samples per individual morphotype from both depths.

5.5 Open Reading Frame (ORF) prediction

Using the EvidentialGene output, we observed that the digitate morphotype contains the most ORFs, including complete ORFs (**Table 1, Table 2**).

5.6 Gene ontology (GO)

Ortholog hits were run in Trinotate against three functional annotation classifications: biological processes, molecular function and cellular components. All transcriptomes had hits over the three classifications (**Figures 5, 6, 7**). Each classification was represented with twenty gene-associated categories. From the top twenty categories transmembrane transport, protein binding and integral component of membrane were found to have the highest functional annotations in biological processes, molecular functions and cellular components, respectively.

Genes associated with biological processes revealed similar number of sequences across all samples, with transmembrane transport and oxidation-reduction process as the highest categories within this classification (**Figure 5**).

In cellular components (**Figure 6**), the first two categories contained more annotated sequences than the biological classification. However, the Biological classification had the lowest number of annotated sequences (<1,000).

The last GO classification observed was molecular function (**Figure 7**), which reported the highest number of annotated sequences annotation of all three categories. Genes related to protein binding reported more than 4,500 sequences while genes related to the ion channel activity category reported almost 800 annotated sequences. Still, molecular function represents the category with the highest gene annotation. The combined encrusting *B. asbestinum* samples reported the highest number of sequences annotated in all three main classifications and all eight gene categories. Overall, the highest hits of GO classification were achieved by transcriptome of a morphotype or colony types independent of depth collection.

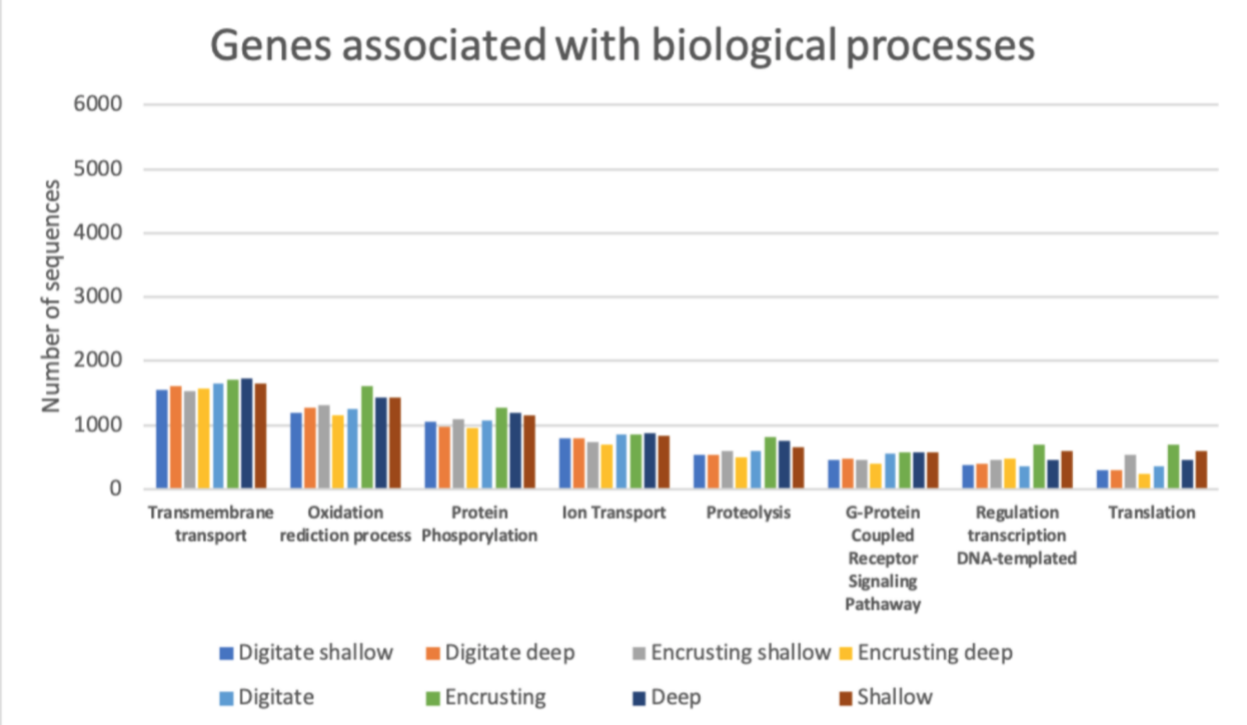

Figure 5: Gene ontology for biological process of *Briareum asbestinum* using Trinotate.

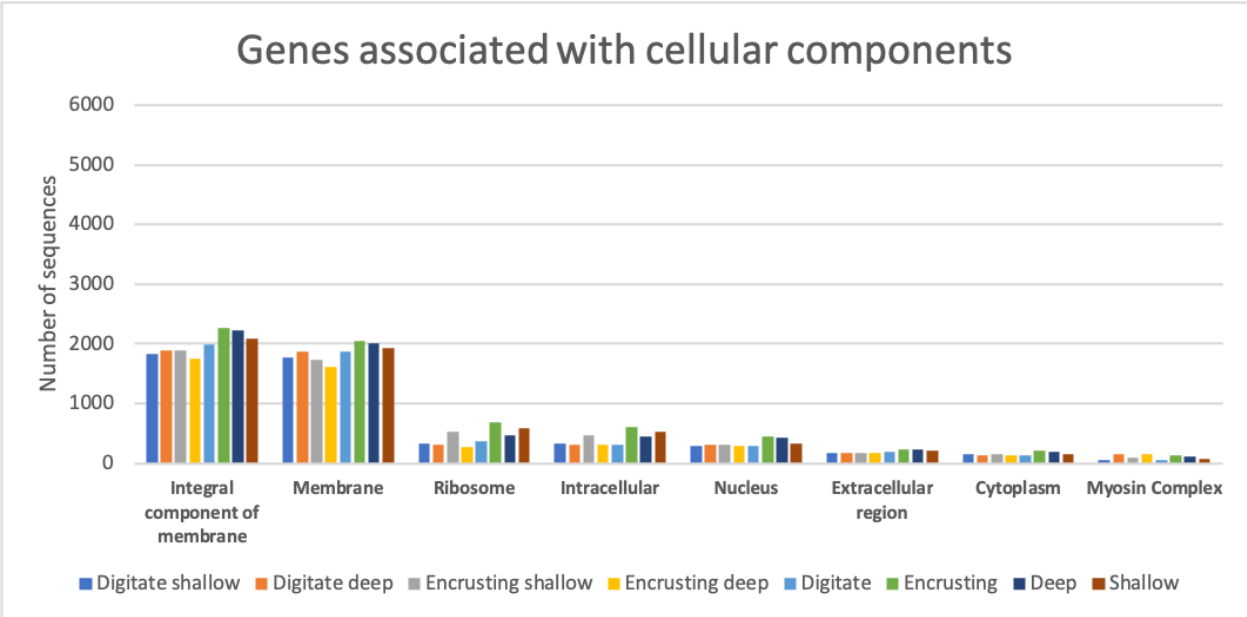

Figure 6: Gene ontology cellular components of *Briareum asbestinum* using Trinotate.

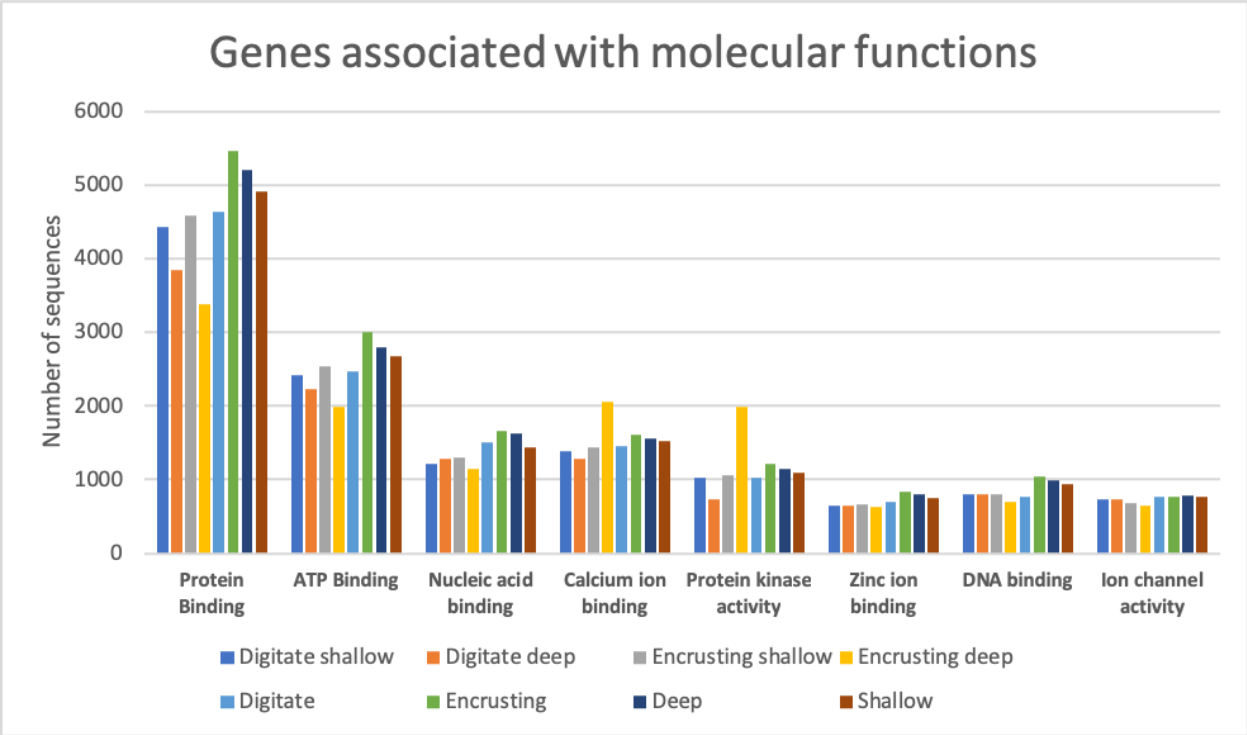

Figure 7: Gene ontology for molecular function of *Briareum asbestinum* using Trinotate.

5.8 Phylogenetic analysis

5.8.1 Concatenation

5.8.1.1 *Heliopora coerulea* as the outgroup

Phylogenetic analysis based on amino acids was conducted using the Maximum Likelihood criterion. This analysis was based on 2,568 orthologous genes when including *H. coerulea*. Results showed a significant sequence divergence between all *Briareum* morphotypes and *Heliopora coerulea*, as expected (**Figure 8**). While shallow samples from both *Briareum* morphotypes formed a sister group, the deep digitate colonies showed the furthest topological relation from all other morphotypes. All branches were supported by 100% bootstrap values.

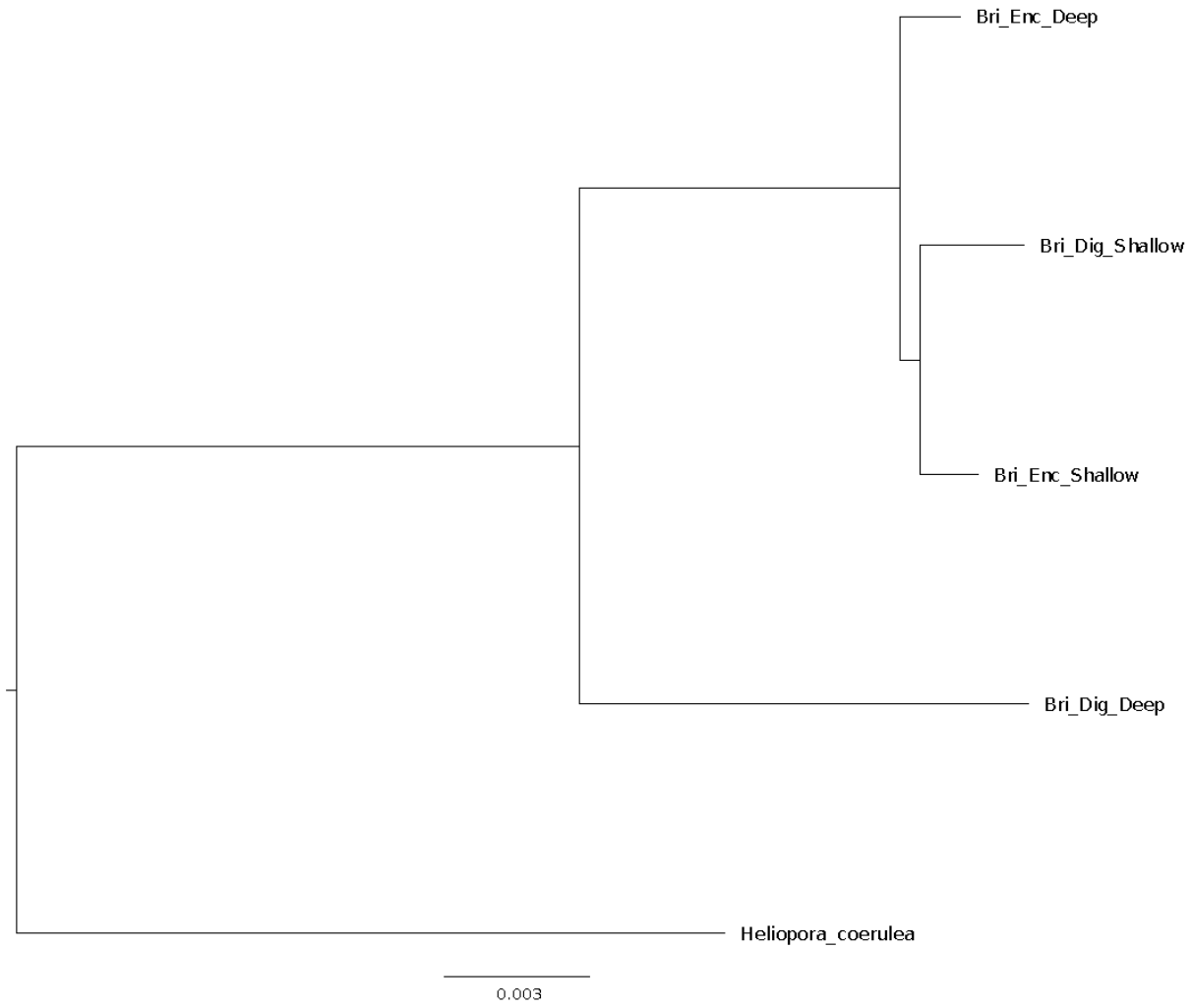

Figure 8: Phylogenetic tree of *Briareum asbestinum* morphotypes based on the Maximum Likelihood method in RAxML-NG with *Heliopora coerulea* as the outgroup. The amino acid substitution model GTR+GAMMA was used as the most appropriate model of evolution. The RAxML-NG program was run using the rapid bootstrapping method search option (-f a) with 1,000 bootstraps.

5.8.2 Coalescence

Maximum Likelihood analysis can produce unrooted trees that serve as input for the coalescent-based species tree estimation program ASTRAL-II. *H. coerulea* was not used in order to avoid higher sequence divergence and higher gene dropouts when using the *Briareum*

datasets. In this phylogenetic tree, similar topologies were generated among the morphotypes as in the concatenated method, except that the *Briareum* encrusting deep colony was recovered as the most phylogenetically distant taxon with the coalescent method (**Figure 9**).

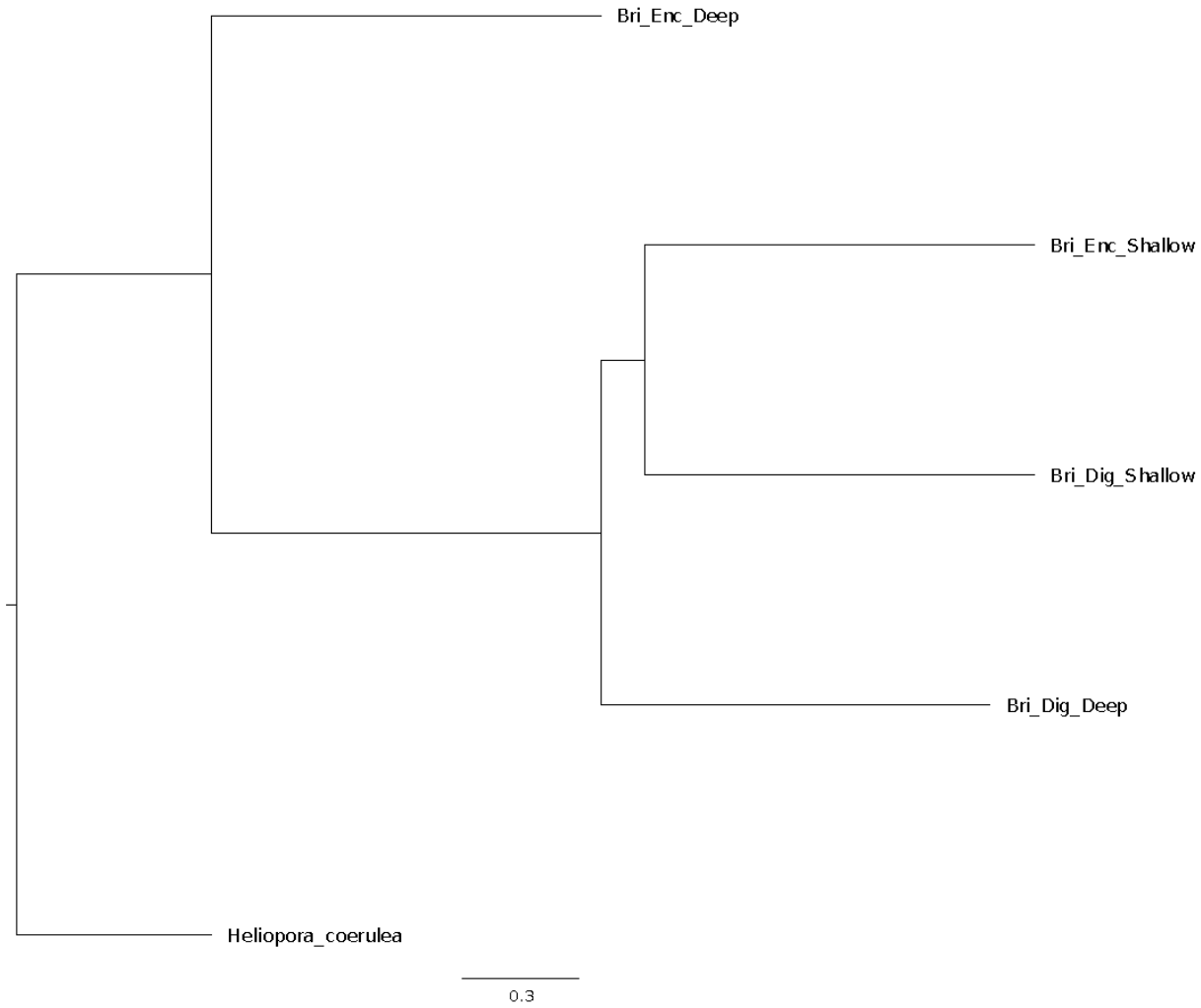

Figure 9: Phylogenetic tree based on the Maximum Likelihood Method as implemented in RAxML-NG. Dataset was constructed with the coalescence approach. RAxML-NG was run for every single gene file with GTR+GAMMA as the substitution model and the rapid bootstrapping method (-f a) with 1,000 bootstraps. The trees were used as input for the program Astral-II and was run with the full annotation mode (-t 4) and 100 replicates. Nodes are supported with 100% bootstrap support unless otherwise noted.

6. Discussion

6.1 Phylogeny

In a recent review on speciation genomics, Campbell et al. (2018) highlights the potential of high throughput genomic data to understand the interacting factors driving to lineage diversification and eventual speciation. The expectations from generating large data sets through high throughput sequencing methods was to resolve recalcitrant nodes, such as those of the *Briareum asbestinum* morphotypes, where traditional approaches have failed to resolve.

We have increased the data set used for phylogenetic analysis of the *Briareum* morphotypes by several orders of magnitude, by generating high quality transcriptomes of *Briareum* colonies sampled from shallow and deep habitats within reefs. However, based on our analysis between both morphologies of *B. asbestinum*, we did not recover reciprocally monophyletic groups based on colony morphology suggesting no clear phylogenetic distinction between them. Instead, regardless the method used (i.e. concatenation vs. coalescence), the phylogenetic analysis recovered a very close relationship between the two morphotypes from shallow reefs. The two morphotypes of *B. asbestinum* have been observed in closer proximity along shallow reefs than in deeper reefs, possibly supporting the closer phylogenetic relationship.

These results agree with previous hypothesis posed from Bilewitch et al. (2010), where they conclude that a variation within a growth form can be taken as evidence that at least some degree of genetic exchange still exists between the digitate and encrusting forms and, at most, the two morphs are at the earliest stages of divergence. All *B. asbestinum* deep colonies were less frequently found in proximity across Beril Reef during sampling. This observation together with the phylogenetic analysis suggests low levels of gene flow within this depth. However, we cannot completely ascribe these results solely based on the resulted topologies. Further analysis on differential expression can be done for these replicates with more details on putative assignments to gene families, potentially related to determination of colonial growth form.

Guzman et al. (2010) found enriched ortholog groups exclusively from *H. coerulea* associated with different gene families, including those in developmental pathways. Jeon et al. (2018) recently published a draft genome of the octocoral *Dendronephyta gigantea*, where Hox gene clusters were found to be different between soft and stony corals. These homeobox genes encode for transcription factors that execute different functions during the organism's development. Moreover, subsequent comparative analysis should be performed for *B. asbestinum* developmental stages, as key genes that may determine the architecture colony may be expressed only during the larval and juvenile stages.

We observed conflicted topological signals between the concatenated and coalescence approaches, where the *Briareum* digitate deep and the *Briareum* encrusting deep were the most phylogenetically distant taxa, respectively. Consensus between the two approaches was reached only in the formation of a well supported clade consisted of the digitate and encrusting forms of the shallow *Briareum* colonies. Phylogenetic discordance between the two approaches is not uncommon and the theoretical framework of each approach is still vigorously debated (e.g. Song et al. 2012; Gatesy and Springer 2013). Phylogenetic conflicts from big data sets can arise when there is inconsistency of phylogenetic estimates, especially in both closely and distantly related taxa. Especially in cases where taxa are very closely related (i.e. *Briareum*), presence of phylogenetic noise due to incomplete lineage sorting (ILS) may be the primary source of topological conflict. In these cases, incorporating of variation in gene histories may improve the analysis in the presence of conflicted phylogenetic signal (Kubatko and Degnan 2007).

6.2 Taxonomic status in *B. asbestinum*

By presenting this first multiple transcriptome assembly of the two morphotypes of *B. asbestinum* we may get a glance at the transcriptional architecture of plasticity. We

observed a distinction between digitate deep or encrusting deep samples, depending on the analysis, and all other shallow samples. However, we did not observe a consistent topological difference between morphotypes across depths. As Fitzpatrick (2012) suggests, phenotypic plasticity might exist even in a homogeneous population in a homogeneous environment. The colonies exhibiting encrusting and digitate morphologies may display local adaptation. In the shallow reefs both morphotypes are readily observed next to each other. However, as depth increases, they are found in different sites. Environmental heterogeneities as light exposure, temperature change and currents may be a factor to consider that favors the isolation of deep colonies promoting genetic differences between morphotypes and between depth (Buskirk 2002). Phenotypic plasticity may not be the sole factor of determining the two morphotypes if they encounter different microhabitats. Oostra et al. (2018) reviewed the state of knowledge on phenotypic plasticity and adaptation and suggested that the conditions under which phenotypic plasticity leads to evolutionary adaptation or not, when the environment changes, are not defined. More detailed gene expression experiments and transplantation of *Briareum* colonies to different environments are required to uncover possible significant genetic components, which determine the architectural design of a colony. Early environmental cues during development may also be an important factor determining the shape of each colony, therefore, ontogenetic gene expression studies may prove to be very useful in *Briareum* and other reef species exhibiting high rates of plasticity (e.g. *Millepora* spp.). An increased understanding of the role of plasticity requires a 'whole organism', rather than 'single trait' approaches, taking into consideration that organisms are integrated complex phenotypes.

7. Conclusions

- We did not recover the two morphotypes of *B. asbestinum* as reciprocally monophyletic groups based on the analysis of the transcriptomic data.
- Regardless the method used (i.e. concatenation vs. coalescence), the phylogenetic analysis recovered a very close relationship between the two *B. asbestinum* morphotypes from shallow reefs.
- We recovered conflicted phylogenetic signals between the concatenated and coalescence approaches, where the *Briareum* digitate deep and the *Briareum* encrusting deep were the most phylogenetically distant taxa, respectively.
- The two morphologies represent phenotypic variability of the same species, *B. asbestinum* and until further evidence is presented to the contrary, *B. polyanthes* is not a valid species.
- The *de novo* transcriptomes presented here are the first genomic resources for the *Briareum* genus.

8. Further studies

- These data sets can serve as a reference for subsequent transcriptome comparisons with the larval and juvenile stage, to understand the transcriptional ontogenetic differences.
- Further analysis on differential expression between shallow and deep reef morphs of *B. asbestinum* to assess possible differences at the expression level rather than the sequences themselves.
- Comparison of gene expression with other octocorals.
- Genome sequencing of *Briareum asbestinum.*

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10. Appendix

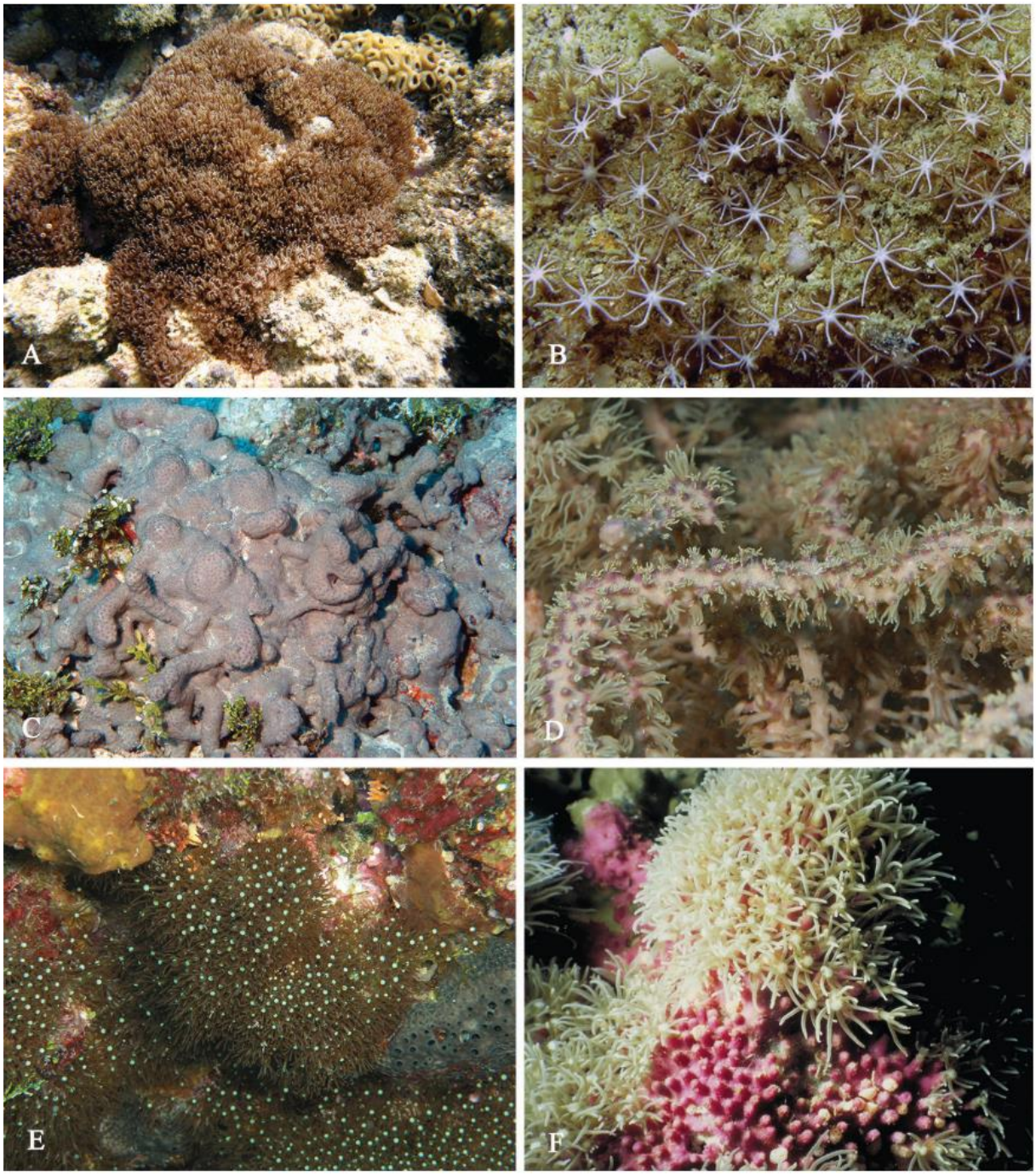
10.1 MIxS

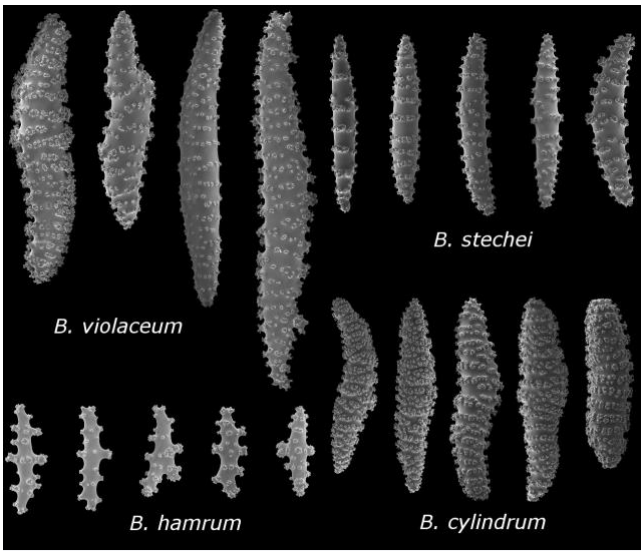
Appendix, Table 3: MIxS data description for the coral *Briareum asbestinum*

Item	Definition
General feature of classification	
Classification	Eukaryota; Opisthokonta; Metazoa; Eumetazoa; Cnidaria; Anthozoa; Octocorallia; Alcyonacea; Scleraxonia; Briareidae; Briareum; *Briareum asbestinum*
Investigation type	Eukaryote transcriptome
Project name	*Briareum asbestinum* transcriptome (digitate morphotype)
Environment	Coral reef
Geographic location	Caribbean Sea,
	Puerto Rico, La Parguera
Latitude, longitude	Turrumote: 17°56'06.4"N, 67°01'06.5"W Beril: 17°52'47.8"N, 67°03'02.7"W
Collection date	2/04/2017
Environment properties	Shallow Reef and Shelf edge
Depth	5 m and 25 m
Collector	Liajay Rivera García
Sequencing	
Sequencing method	Illumina NextSeq 500; Paired-end (2 x 150)
Assembly	
Method	*De novo* assembly
Program	Trinity v2.6.6, SOAPdenovo-Trans, Velvet v1.2.10, Oases v0.2.09, Trans-AbySS, EvidentialGene v2013.07.27
Finishing strategy	High quality transcriptome assembly
Accessibility	
DDBJ/ENA/GenBank	GHBD00000000

11. Pictures

11.1 *Briareum* Indo Pacific colonies





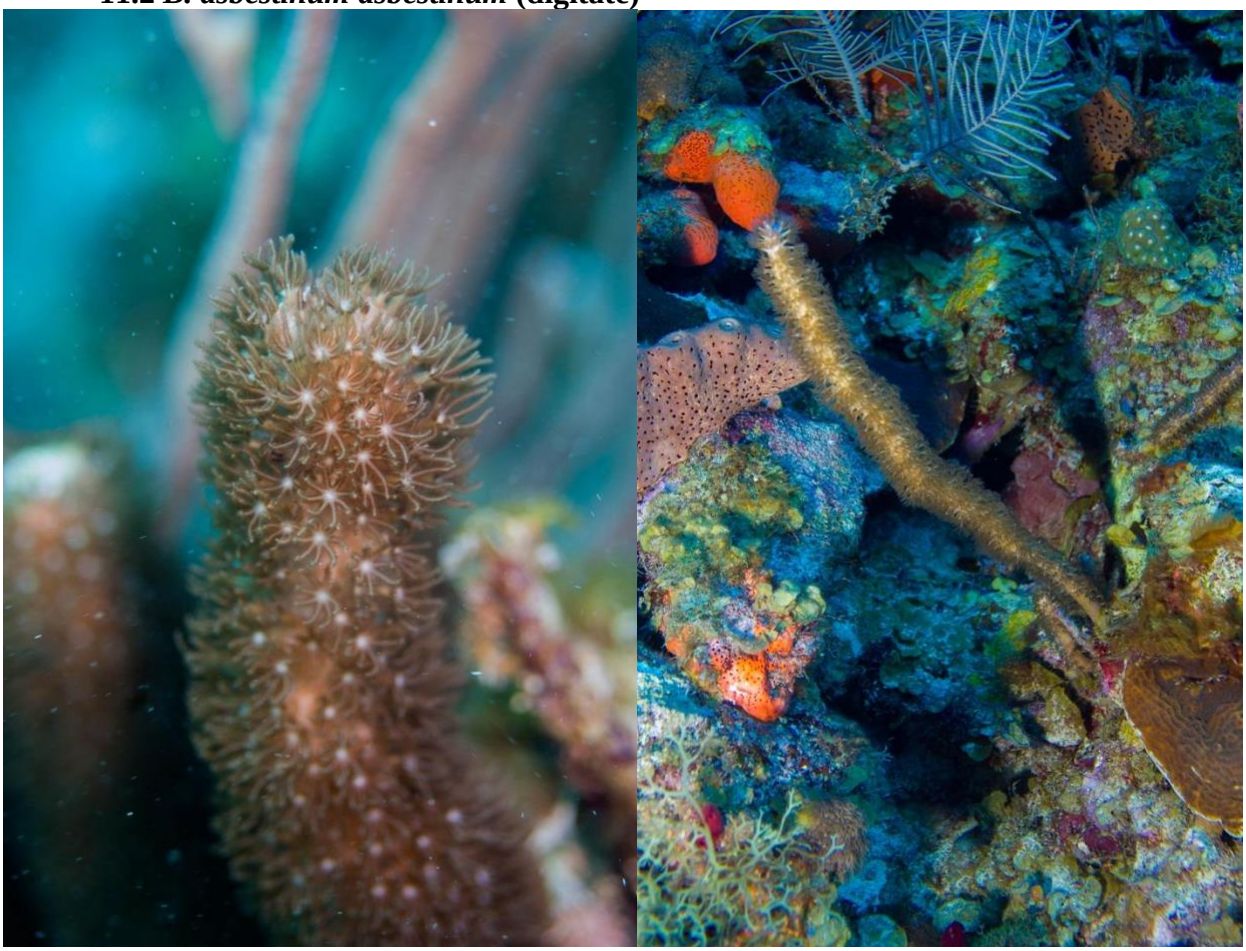
Appendix, Figure 10A: **A–B)** *Briareum hamrum* **A)** Colony **B)** close-up of tentacles **C–D)** *Briareum stechei* **C)** Colony **D)** close-up of tentacles **E–F)** *Briareum violaceum* **E)** Colony **F)** close-up of tentacles **G)** Sclerites of all Indo-Pacific *Briareum* species.

11.1 *Briareum asbestinum* (encrusting)

Appendix, Figure 11A: *Briareum asbestinum* encrusting shallow sample from Turrumote Reef, La Parguera, Puerto Rico.

Appendix, Figure 11B: *Briareum asbestinum* encrusting deep sample from Beril Reef, La Parguera, Puerto Rico.

11.2 *B. asbestinum asbestinum* (digitate)



Appendix, Figure 12: (Left) *Briareum asbestinum* digitate shallow sample from Turrumote Reef, La Parguera, Puerto Rico. (Right) *B. asbestinum* digitate deep sample from Beril Reef, La Parguera, Puerto Rico.