POPULATION GENETICS OF BRITTLE STARS IN THE GULF OF MEXICO. Abbey Hebert[1*], Shelby Shaw[1*], and Walter Cho[1], [1]Department of Biology, Point Loma Nazarene University, San Diego, CA 92106.

Abstract

A population genetic study of brittle stars was conducted in the Gulf of Mexico to study patterns of gene flow and population structure. Mitochondrial DNA from 85 samples was extracted, amplified, and sequenced using 16S and COI primers. Phylogenetic analyses confirmed the identity of three species, *Asteroschema clavigerum* and two species exhibiting cryptic speciation: *Ophiocreas spinulosus* and an unidentified *Asteroschema* sp. 2. Nested clade phylogeographic analysis and analysis of molecular variance showed that only *O. spinulosus* had significant genetic structure based on geography or depth and potential isolation by distance. *O. spinulosus* and the unidentified *Asteroschema* sp. 2 both showed high levels of gene flow and no population structure. Research of population connectivity in marine species with anthropogenic disturbances is important in understanding species conservation and the effects of habitat change and pollution.

Introduction

The Gulf of Mexico, an ocean basin mainly surrounded by the North American continent, is a largely biodiverse area, home to many aquatic species. Being such a large area, it has differing climates, with the northern areas considered to be warm-temperate, and the southern areas considered to be tropical (Niegel, 2009). The specific areas of interest in this study are the deep-sea regions of the Gulf of Mexico. These regions are of great concern, due to the increase of anthropogenic disturbances, most notably the Deepwater Horizon oil spill in 2010 (Quattrini et al., 2013).

The focus of this study is the brittle star, an aquatic organism which is a part of the class Ophiuroidea, in the phylum Echinodermata (Pawson et al., 2009). Ophiuroids make up the largest class within the phylum Echinodermata, with over 1900 extant species (Tyler, 1980). A unique characteristic of the brittle star is that they utilize various reproductive strategies, two of the most common being brooding and spawning. Brooding is when the individuals will keep the gametes within their bodies for fertilization, whereas spawning involves sending the gametes out

into the water to be fertilized. This is prevalent in this study, as within the Gulf of Mexico there is a loop current that could have an impact on the dispersal of gametes released by spawning brittle stars, and thus influence gene flow.

The Gulf of Mexico is home to around 7% of all echinoderms in the world, making it an ideal location for this study (Pawson et al., 2009). Brittle stars, like other echinoderms, are considered to be benthic animals, which means they live on, in, or near the seabed, which is called the benthic zone (Pawson et al., 2009). They are generally found in deep-sea habitats, but they have a wide bathymetric range (Tyler, 1980). In their deep-sea habitat, brittle stars live in the benthos or can live in association with other organisms like sponges or corals (Cho and Shank, 2010).

Brittle stars have been known to show cryptic speciation in the past, meaning that it may be difficult to differentiate their species based on morphological characteristics alone. The fact that ophiuroids are considered to be cryptic species is evident in their observed level of variation in phenotypic characteristics, even within populations (Baric and Sturmbauer, 1999). Previous studies have shown that determining the lineages of ophiuroids can be challenging. For example, a study of Mediterranean ophiuroids found that individuals thought to belong to the species *O. quinquemaculata* split into two different lineages (Baric & Sturmbauer, 1999). Additionally, individuals that were previously considered to be distinct species actually had identical genotypes (Baric & Sturmbauer, 1999).

Due to their cryptic nature, molecular analysis is an effective method to differentiate between different species of brittle stars. In this study we utilized two different mitochondrial DNA (mtDNA) markers to resolve species identity and perform a population genetic analysis investigating population structure and gene flow among brittle star populations in the Gulf of Mexico.

The Gulf of Mexico has rich evolutionary diversity, as well as a significant number of endemic species (Niegel, 2009). Previous studies of population structure in the Gulf of Mexico have been largely related to taxa which serve commercial or recreational purposes, which has left many species understudied (Niegel, 2009). The lack of comprehensive studies of brittle stars can be partially attributed to this fact. One study, which focuses on octocoral community structure, sheds light on the population structure of species in the Gulf of Mexico (Quattrini et al., 2014). This study is particularly relevant, as brittle stars are associated with octocorals. Studying deep-

water octocorals using both morphological and molecular data is useful to discern patterns of biodiversity within the Gulf of Mexico, as well as obtain a deeper understanding of the biogeographic nature of the area (Quattrini et al., 2014). It was found that bathymetry was an important factor for community structure, which led us to consider the impact of bathymetry on the population genetics of the brittle stars in our study.

Previous studies of brittle stars in different areas of the world have been influential to this study. One study aimed to discern the level of genetic connectivity between North Atlantic seamounts with a focus on four ophiuroid species (Cho & Shank, 2010). The nature of ophiuroids as coral associates was focused on in this study by analyzing the levels of gene flow and how host specificity impacts genetic structure, and in this case it was determined that gene flow was present for all four species, although some genetic structure based on geography and depth was detected for some species (Cho & Shank, 2010). This provided a basis to examine gene flow of the brittle stars in our study. A second study focused on brittle star populations in New Zealand, with the goal to determine how the physical environment and biogeography impact genetic diversity (Sponer et al., 2001). It was found that various environmental factors impacted the genetic diversity of the brittle star populations, most predominantly ocean currents and water temperatures (Sponer et al., 2001). The Gulf of Mexico has varying water temperatures and a significant loop current, both of which may play a role in the population structure of the brittle star communities inhabiting the area.

Materials and Methods

Sample collection

Samples were collected in the Gulf of Mexico by the remotely operated vehicle (ROV) Jason II during the TDI-Brooks R/V Ron Brown/Jason2 Lophelia II expedition (August 6 to September 12, 2009 and October 15 to November 1, 2010) and an industrial ROV on board the R/V Holiday Chouest during the non-Lophelia II HC3 research expedition (October 1 – 24, 2011). Collection depths were between 260m and 2600m (Table 1).

After collection, the specimens were placed in insulated bio boxes, before being processed on board, where the arms of each of the ophiuroid specimens were cut off into 2-3 cm samples and frozen at -80°C for later genetic analysis. The remnants of the animal specimens were preserved by placing them in 70% ethanol or freezing them at -80°C.

DNA Processing

DNA was extracted from the ophiuroid samples using a modified 5% Chelex extraction protocol (Walsh et al., 1991; Spencer & Roy, 2002; Cho & Shank, 2010). Briefly, 1-3 mm of arm tissue from each sample was excised, minced, and added to 500 µL of the 5% Chelex solution (Bio-Rad, Hercules, CA), then heated at 65°C for 3 hours. After the 3 hours, the solution was vortexed, heated to 95-100°C for 10 minutes, then centrifuged for 10 minutes at 13,500xg. These DNA extractions were stored at various temperatures depending on length of storage: 4°C or -20°C for short-term storage and -80°C for long-term storage. A total of 106 individuals from 14 Gulf of Mexico sites were collected and processed in the manner described above (Figure 1 and Table 1).

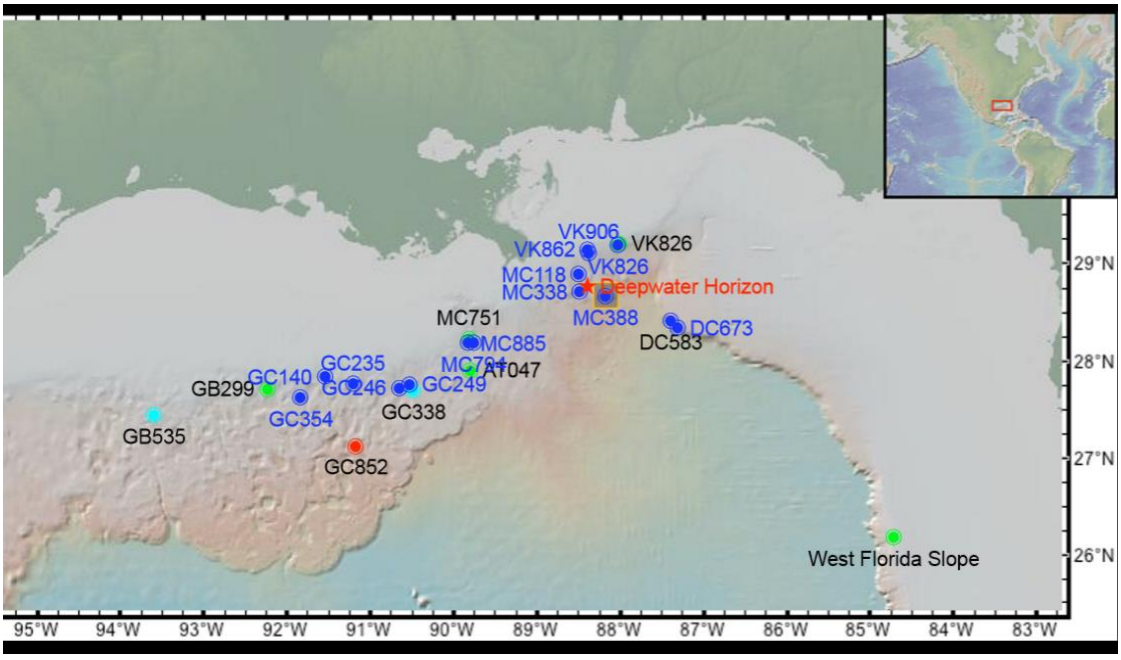

Figure 1: Map of Gulf of Mexico sampling sites.

Table 1: Depth, latitude, and longitude of Gulf of Mexico sample collection sites.

Site	**Depth (m)**	**Latitude**	**Longitude**	**Radius of collection area**	***Ophiocreas spiunlosis***	***Asteroschema* sp. 2**	***Asteroschema clavigerum***
GB299	360.18-365.53	27.6864172°	-92.2306423°	1500	10	6	0
MC751	437.23-441.09	28.1946347°	-89.7990027°	50	3	4	0
MC885	626.00-632.00	28.066485	-89.714745°	178	5	0	0
GC140	259.7-320.44	27.8116721	-91.536578°	162	1	2	0
GC246	848	27.689721	-90.644962°	100	4	0	0
GC249	777.5-790.32	27.72413712	-90.51418108°	1650	5	0	0
VK826	540.19-542.69	29.1546575	-88.0225573°	50	11	0	0
MC118	883.99-887.83	28.85267684	-88.49191865°	50	1	0	0
DC673	2312.7	28.310948	-87.3065322°	1000	0	0	7
MC338	1370.17-1372.5	28.67241779	-88.4765542°	50	0	0	5
MC159	897	28.787224	-88.634678°	50	8	0	0

Table 1. continued

Site	**Depth (m)**	**Latitude**	**Longitude**	**Radius of collection area**	***Ophiocreas spiunlosis***	***Asteroschema* sp. 2**	***Asteroschema clavigerum***
GB299	360.18-365.53	27.6864172°	-92.2306423°	1500	10	6	0
MC751	437.23-441.09	28.1946347°	-89.7990027°	50	3	4	0
MC885	626.00-632.00	28.066485	-89.714745°	178	5	0	0
GC140	259.7-320.44	27.8116721	-91.536578°	162	1	2	0
MC297	1560	28.682045	-88.345226°	400	0	0	4
				Total # of Individuals	52	12	42

DNA Processing:

DNA extractions were diluted with Milli-Q water to a final concentration of 10-20 ng/μL for processing by first determining the concentration of each extraction using a NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, Waltham, MA). Once these dilutions were prepared, they were amplified using Polymerase Chain Reaction (PCR), which contained 5 µL 5X PCR Buffer, 2.5 mM of $MgCl_2$, 0.5 µM of each primer, 0.2 mM of dNTP, 1 µL of extracted DNA template and 0.2 µL of Taq polymerase (Promega, Madison, WI). In addition, 2.5 µL of 5M Betaine (ThermoFisher Scientific, Waltham, MA) was added to 16S PCR reactions in order to increase product yield. Different programs were used for the two primers, 16Sar/16Sbr (Sponer et al., 2001) and COI30/COI31 (Okanishi & Fujita, 2013) (Table 2).

After completion of each PCR reaction, gel electrophoresis was performed using 1.5% agarose gels in order to determine whether or not the PCR reaction was successful. A successful reaction was indicated by the presence of banding in the expected base pair region of the gel. Gels were imaged using ChemiDoc Touch Imaging System (ThermoFisher Scientific, Waltham, MA).

Successfully amplified PCR reactions were then purified using either the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) or Wizard PCR Preps DNA Purification System (Promega, Madison, WI).

Following purification, a second gel electrophoresis was performed to determine whether or not the purification was successful. If successful, the purified samples were sent out for bi-directional Sanger Sequencing at an outside laboratory (Eurofins Genomics, Louisville, KY).

Table 2: PCR conditions for primer sets used in this study.

Primer set	PCR thermocycler program
COI030/031 (COI_50)	1. 94°C-2 mins 2. 94°C-1 min 3. 50°C-1 min 4. 72°C-45 sec Repeat steps 2-4 30 times 5. 72°C-7 min 6. 4°C–∞
COI030/031 (COI_52)	1. 94°C-2 mins 2. 94°C-1 min 3. 52°C-1 min 4. 72°C-45 sec Repeat steps 2-4 30 times 5. 72°C-7 min 6. 4°C–∞

Table 2. continued

COI030/031 (COI_47)	1. 94°C-7 mins 2. 94°C-30 min 3. 47°C-1.5 min 4. 72°C-45 sec Repeat steps 2-4 30 times 5. 72°C-10 min 6. 4°C–∞
16SarL/brH (16S_45)	1. 94°C-2 min 2. 94°C-1 min 3. 45°C-1 min 4. 72°C-45 sec Repeat steps 2-4 30 times 5. 72°C-7 min 6. 4°C–∞
16SarL/brH (16S_45-2)	1. 94°C-5 min 2. 94°C-1 min 3. 45°C-1 min 4. 72°C-45 sec Repeat steps 2-4 29 times 5. 72°C-7 min 6. 4°C–∞

Population Genetics Analysis:

Sequencing results were received from Eurofins Genomics as chromatogram files and downloaded into CodonCode Aligner (CodonCode Corporation, Centerville, MA), which gives

users the ability to check and edit base pair calls within the given sequence. Contigs were made from forward and reverse sequences of the same individual. Ambiguous base calls were resolved by eye and messy ends were trimmed to ensure clean sequence reads. Alignments were made separately for 16S and COI within the CodonCode Aligner program and were checked by eye. Since COI is a protein-coding gene, COI sequences were translated into amino acid sequences in order to confirm the absence of stop codons and ensure the absence of pseudogenes. Once checked, the final alignments for 16S and COI were exported as FASTA files and then concatenated to form a single sequence. This final sequence was used in all the further steps of genetic analysis besides the phylogenetic analysis.

Phylogenetic Analysis:

To confirm the species identity of the samples, a neighbor-joining phylogenetic tree was created using the Kimura-2 parameter model of evolution in the software package MEGAX using the COI alignment (Kumar et al., 1994). Representative samples from each clade were checked against sequences in GenBank using a BLAST search to check for highly similar sequences already present in the database. A new alignment was then created that included the matching BLAST results and representative outgroups using MUSCLE (Edgar, 2004). New alignments were made in MEGAX and checked by eye. MEGAX was then used to create a Neighbor-Joining tree using the Kimura 2-P model of evolution and 500 bootstraps. Pairwise distances between the species and within clades was also calculated using MEGAX, translating differences into distances (Brinkman, 2001).

Haplotype network and NCPA:

Haplotype networks were created separately for each species using the concatenated 16S and COI dataset in the program TCS 1.21 (Clement et al., 2000). The final networks were stylized in TCS beautifier (Santos et al., 2016) to indicate the haplotype frequency by proportional size and geographic location by color. Each site was indicated by consistent colors within the three species. Nested clade phylogeographic analysis was then performed with the software ANeCA 1.2 to test geographic and evolutionary mutations (Panchal, 2007).

Analysis of molecular variance (AMOVA):

Analysis of molecular variance, or AMOVA, was completed with the software ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Comparisons were informed by the results from the nested clade analysis. Separate AMOVA were performed for each separate species. Factors tested among each species included geography (East vs. West) and depth (shallow vs. deep). (Table 1).

Isolation by distance:

A mantel test (Mantel, 1967) was also performed for each species to test for possible isolation by distance in ARLEQUIN 3.5 (Excoffier and Lischer, 2010).

Results

Population Genetics Analysis

An alignment of 16S sequences was created 457 bp in length with a total of 56 individuals for *Ophiocreas spinulosus*, 13 individuals for *Asteroschema* sp. 2, 29 individuals for *Asteroschema clavigerum*. A COI alignment was created being 664 bp in length with a total of 47 individuals for *Ophiocreas spinulosus*, 14 individuals for *Asteroschema* sp 2, and 29 individuals for *Asteroschema clavigerum*.

The 16S and COI sequences were then concatenated and an alignment of 16S and COI sequences was created 1098 bp in length with a total of 46 individuals for *Ophiocreas spinulosus*, 12 individuals for *Asteroschema* sp 2, and 27 individuals for *Asteroschema clavigerum*. Some individuals ended up being excluded in the concatenated sequence because they were too short, or their sequence was too ambiguous when compared to other chromatograms.

Phylogenetic analysis:

The phylogenetic tree based on the COI dataset created using MEGA recovered five distinct clades (Figure 2). The average within clade genetic distance was calculated to determine species identity (Table 3). All values fell below the 3% sequence divergence cut-off typically associated with separate species in brittle stars (Sponer, R., et al., 2001). The average between

distance clades was also calculated with values ranging from 0.117-0.292, all above the 3% sequence divergence threshold for species indicating that each clade is a separate species (Table 4). Three distinct species were identified*: Ophiocreas spinulosus*, a second unidentified species, and *Asteroschema clavigerum* (Figure 2). In addition to this, an outgroup species was also identified (*Ophioctenella acies* isolate). Sample AU.GC140.04_1 was identified as a separate species, *Asteroschema oligactes* (based on the 16S dataset, data not shown).

Figure 2: Neighbor-joining tree using the COI dataset resulted in 5 clades, with four clades identified to species. The Kimura-2 parameter was used with a distance and a bootstrap of 500

(Kimura, 1980). In later analyses, *Asteroschema oligactes* and *Ophioctenella acies isolate* (outgroup) were excluded.

Table 3: Pairwise distances within the clades shows the level of divergence within each species' clade. Sample GC140.04 did not have a calculated value because it could not be compared to itself. A total of 93 sequences were used. The number of base substitutions per site from averaging over all sequence pairs within each group are shown. The software removes ambiguous positions for each sequence pair.

Average within Clades - Genetic Distance	
Ophiocreas spinulosus (Sp1)	0.03
Species 2	0.00
Asteroschema clavigerum (Sp3)	0.01
Asteroschema oligactes (GC140.04)	N/C
Ophioctenella acies isolate Outgroup)	0.00

Table 4: Pairwise distances between the clades shows the genetic distance as well as the level of divergence between the clades. The number of base substitutions per site from averaging over all sequence pairs between groups are shown. Analysis involved 93 nucleotide sequences and 306 positions in the final data set. Ambiguous positions were removed for each sequence pair.

Average Between Groups	*Ophiocreas spinulosus (Sp1)*	*Species 2*	*Asteroschema clavigerum (Sp3)*	*Asteroschema oligactes (GC140.04)*	*Ophioctenella acies isolate Outgroup)*
Ophiocreas spinulosus (Sp1)	0				
Species 2	0.117	0			
Asteroschema clavigerum (Sp3)	0.163	0.184	0		
Asteroschema oligactes (GC140.04)	0.151	0.156	0.224	0	
Ophioctenella acies isolate Outgroup)	0.247	0.261	0.185	0.292	0

Haplotype networks and NCPA:

The haplotype network for *Ophiocreas spinulosus* included 46 samples distributed in nine haplotypes. The ancestral haplotype was identified as in Clade 1-3 (see Figure 3). Haplotypes unique to sites GB299 and GC140 were separated from the other haplotypes by at least 5 mutations (see Clade 2-1 in Figure 3). This separation was supported by the NCPA that found two significant results: allopatric fragmentation within the total cladogram and restricted gene flow with isolation by distance for clade 1-3.

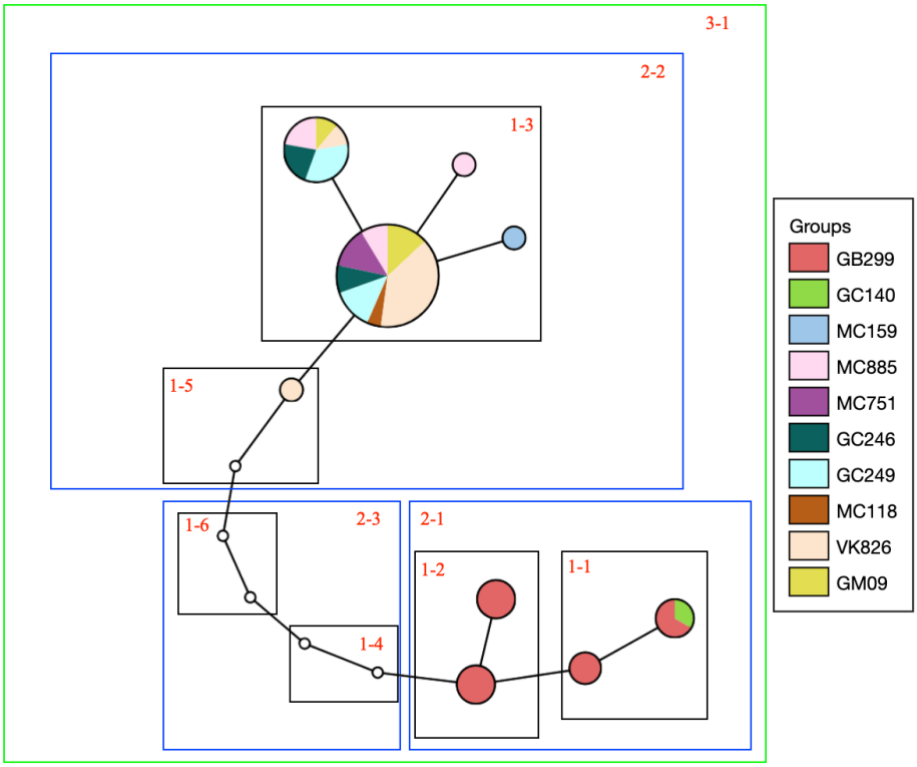

Figure 3: Haplotype network of *Ophiocreas spinulosus.* The colors within each haplotype represent the site and the size of the circles is proportional to the number of individuals within each given haplotype. Each step represents a single mutation. The ancestral node is depicted by the largest circle. The black boxes represent nesting level 1, the blue boxes represent nesting level 2, and the green boxes represent nesting level 3.

The haplotype network for *Asteroschema* sp. 2 included 12 samples distributed in two haplotypes. The ancestral haplotype was identified as the most abundant haplotype as seen in Clade 1-1 (See Figure 4). NCPA found no significant results.

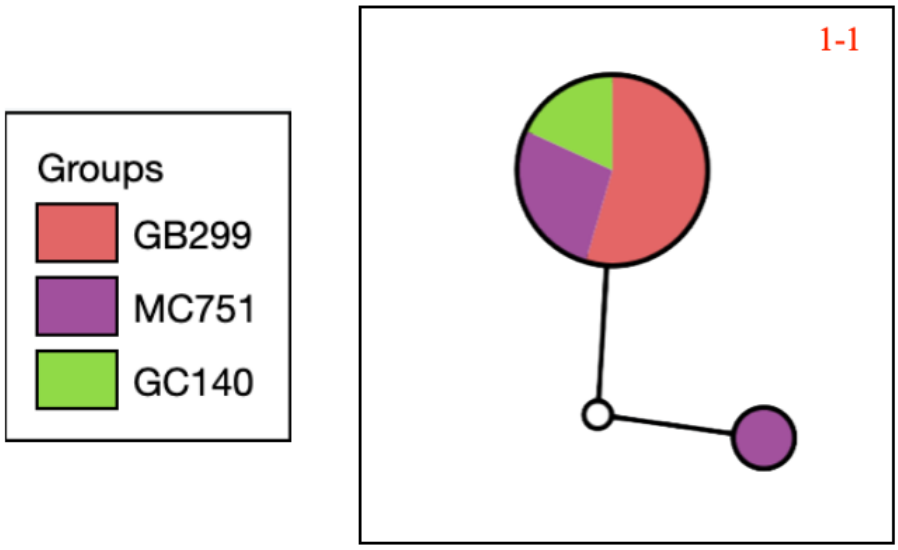

Figure 4: Haplotype network of *Asteroschema* sp. 2. The colors of the nodes represent the site, and the size of the circles is proportional to the number of individuals within each given haplotype. Each step represents a single mutation. The ancestral node is depicted by the largest circle.

The haplotype network for *Asteroschema clavigerum* included 27 samples from four sites distributed in 17 haplotypes (Figure 6). The ancestral haplotype was identified as the most abundant haplotype (See Clade 1-4 in Figure 5). NCPA found that clade 1-3 showed possible range expansion.

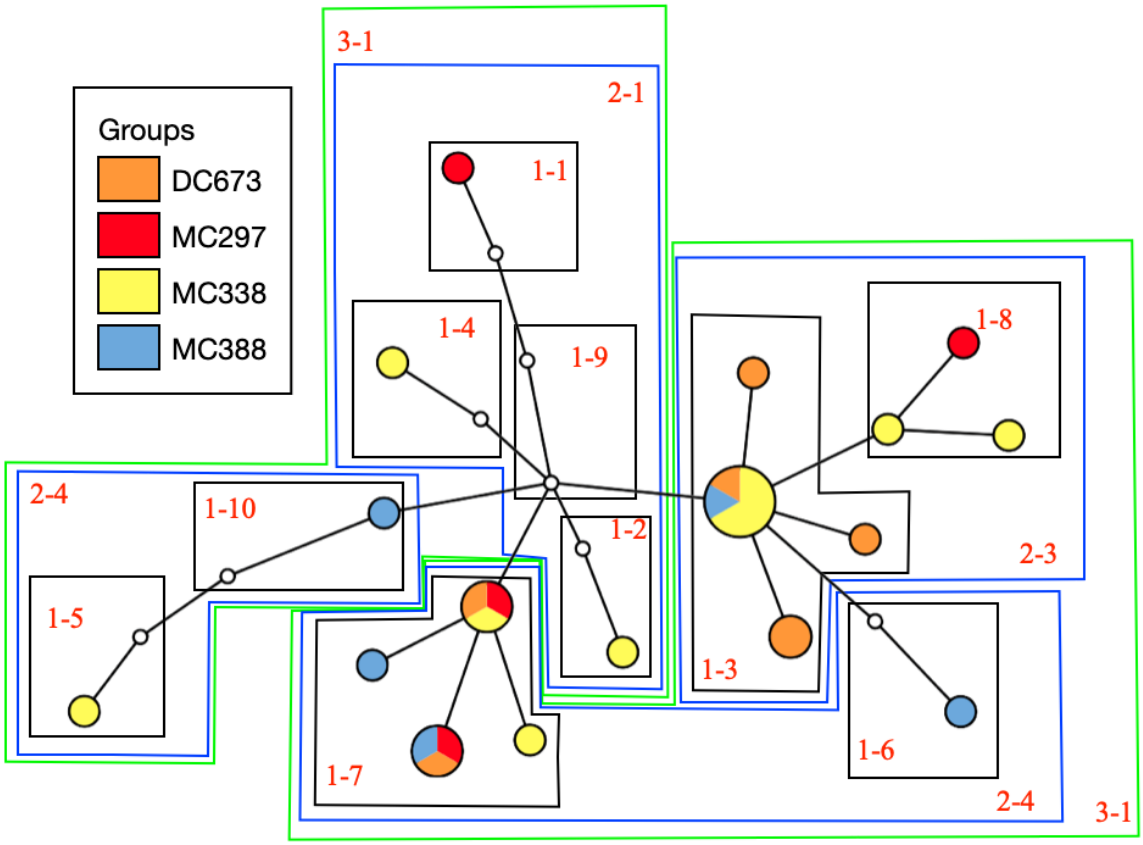

Figure 5: Haplotype network of *Asteroschema clavigerum.* The colors of the nodes represent the site and the size of the circles is proportional to the number of individuals within each given haplotype. Each step represents a single mutation. The ancestral node is depicted by the largest circle (see clade 1-3). The black boxes represent nesting level 1, the blue boxes represent nesting level 2, and the green boxes represent nesting level 3.

Analysis of Molecular Variance (AMOVA):

Multiple comparisons were made to test the factors of geography and depth that may impact population structure for all three species: 1.) A comparison based on geography was made with Eastern sites (VK826, MC118, MC159, GC338, GC249, GC246, MC885) versus Western sites (GB299, GC140, GC235, MC751) (see Figure 1); 2.) a comparison based on depth was made with shallow sites (GB299, GC140) vs. deeper sites (VK826, MC118, MC159, GC338, GC249, GC246, MC885, GC235, MC751).

For *Ophiocrease spinulosus*, significant population structure was found for both comparisons (Table 5). Depth comparison resulted in 90.49% of the variation being accounted for in comparison among groups versus while the geography comparison resulted in 56.13% of the variation accounted for among groups. In addition, both geography and depth may be confounding factors as GB299 and GC140 are both the westernmost and shallowest sites.

No significant results were found for *Asteroschema* sp. 2 or *Asteroschema clavigerum* ($p>0.05$ for all comparisons, not included).

Table 5: AMOVA results for *Ophiocreas spinulosus* for samples organized by geography and samples organized by depth.

By Geography: Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	Significance
Among groups	1	37.060	1.49083 Va	53.13	P-value = 0.00000+-0.00000
Among populations within groups	9	28.839	0.79541 Vb	29.95	P-value = 0.00000+-0.00000
Within populations	35	12.936	0.36961 Vc	13.92	P-value = 0.03324+-0.00381
Total	45	78.836	2.65585		

By Depth: Source of Variation	d.f.	Sum of Squares	Variance Components	Percentage of Variation	Significance
Among groups	1	61.657	3.65390 Va	90.49	P-value = 0.00000+-0.00000
Among populations within groups	9	3.773	0.01473 Vb	0.36	P-value = 0.14467+-0.01255
Within populations	35	12.918	0.36909 Vc	9.14	P-value = 0.01173+-0.00304
Total	45	78.348	4.03772		

Isolation by distance:

Mantel test results were not significant with $p>0.05$ for all three species (Table not included).

Discussion

Phylogenetic analysis confirmed the presence of five distinct species within the 87 samples: *Ophiocrease spinulosus*, *Asteroschema* sp. 2, *Asteroschema oligactes*, *Asteroschema clavigerum*, and *Ophiactinella actes* (Figure 2, Tables 3 and 4). All species including the outgroup came back with high percent identity except for *Asteroschema* sp. 2. The second species in this group was not positively identified, although it matched closest with *Ophiocreas spinulosis* with a sequence divergence of 11.7%. This is unsurprising, as individuals of both *Ophiocreas spinulosus* and *Asteroschema* sp. 2 were both initially identified as the same morphospecies, indicating cryptic speciation as has been observed in brittle star populations prior (Baric and Sturmbauer, 1999). Interestingly, while individuals of both *Ophiocreas spinulosus* and *Asteroschema* sp. 2 were co-occurred and were collected from the same sites, (GC299, GC140, and MC751) these sites were the three shallowest sites in the study (depth < 450 m), perhaps indicating that *Asteroschema* sp. 2 may be restricted to shallow sites. The results of NCPA, AMOVA, and the mantel test were relatively consistent with each other. Both NCPA and AMOVA found evidence of significant population structure for *Ophiocreas spinulosus*. While NCPA found support for isolation by distance for some of the populations mostly located in the Eastern part of the Gulf of Mexico, this was not supported by the Mantel test.

No significant results were found for *Asteroschema* sp. 2 from NCPA, AMOVA, and the mantel test, therefore indicating high levels of gene flow and no population structure. It is important to note, however, that the sample size was relatively small for this species, with only 12 individuals from three sites, all located in the shallowest sites in the western Gulf of Mexico. Analysis of more samples may help to further elucidate the population structure for this species.

Analysis of *Asteroschema clavigerum* showed limited population structure. NCPA found support for range expansion for a sub-clade of the haplotype network. Results from AMOVA and the mantel test were both not significant indicating high levels of gene flow between the populations. This may not be surprising as this species is known to extend out to the Northern Atlantic Ocean (Cho and Shank, 2010) and so it may be capable of long-distance dispersal. Also,

samples were collected from four sites, DC673, MC297, MC 388, and MC338. All were from the deepest sites, 1500-2400m and geographically the MC297, MC388, and MC338 sites were relatively close to each other. The lack of structure may be due to the limitations of sampling and more extensive sampling may provide a clearer understanding of the population structure of this species.

The findings in this study relate back to the overall question of how the different species of brittle stars within the Gulf of Mexico relate to each other and the level of gene flow within them, between them, and among them. From the results of our analysis, it was confirmed that three species exist among the sites that samples were gathered from in the Gulf of Mexico. The results for species 1, *Ophiocreas spinulosus*, showed significant results for samples separated by both geography and depth. Through both NCPA and AMOVA, species 2 showed no pattern of genetic structure or gene flow. A Mantel test concluded that there was no correlation between geographic distance and gene flow (Diniz-Filho, José Alexandre F. et al., 2013). For species 3, *Asteroschema clavigerum*, NCPA and AMOVA showed no significant gene flow or structure.

From Figure 1 and Figure 2, a source of possible gene flow may come from geographic location or geographic depth. Due to their ability to move, brittle stars can either rely on sexual reproduction or brooding (Eaves, Alexandra A., 2003). The location and depth of the samples could make it easier or harder for gametes of different species to join together. The current loop in the Gulf of Mexico could also be a cause of gene flow due to its regular pattern of water flow. Within our study, we found that none of the observed species had isolation by distance, so gene flow may be regular.

The specific samples used within this study were collected from the Gulf of Mexico during the years of 2008 and 2010. This means that we have samples from both before and after the Deepwater Horizon oil spill on April 20, 2010 (Quattrini et al., 2013). Population genetic studies of echinoderm brittle stars within the Gulf of Mexico have not been completed prior to this project, and as such, this data provides a baseline. Data collection and analysis techniques used in this study can be used in similar circumstances to further investigate biodiversity in areas impacted by pollution or human activity. Understanding the connectivity of marine populations is of growing importance, especially for conservation efforts as human impact on the marine environment continues to increase, such as the Orange County Oil Spill in October 2021 (NASA,

2021). In populations that experience high levels of pollution, such analyses could show how different species and populations are impacted over time and how they may be conserved.

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