Mitogenomic phylogenetic analyses of Leptogorgia virgulata and Leptogorgia hebes (Anthozoa: Octocorallia) from the Gulf of Mexico provides insight on Gorgoniidae divergence between Pacific and Atlantic lineages

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INTRODUCTION

There are 54 valid species in the genus *Leptogorgia* belonging to the family Gorgoniidae (Milne-Edwards & Haime, 1857). They are classified as soft corals due to their lack of a protective calcium carbonate exoskeleton. Instead, for support and protection, they rely on small, calcitic structures called sclerites (O'Neal & Pawlik, 2002), from which their white, translucent polyps protrude, and they range in color from yellow to orange to red to purple (White & Strychar, 2010).

*Leptogorgia* and other octocorals provide habitat heterogeneity and therefore allow for large aggregations of diverse fauna (Quattrini et al., 2014). Greater habitat complexity has been shown to be significantly correlated with higher red snapper abundance, an economically important fish species in the Gulf of Mexico and Western Atlantic Ocean (Szedlmayer, 2007). There are nine species of *Leptogorgia* in the Gulf of Mexico, including *Leptogorgia hebes* and *Leptogorgia virgulata* (Cairns & Bayer, 2009). They are found at depths ranging from 2 to 309 m, with the depth range of *L. hebes* ranging from 9 to 37 m and that of *L. virgulata* from 3 to 82 m (Cairns & Bayer, 2009; Williamson, Strychar, & Withers, 2011). *Leptogorgia hebes* and *L. virgulata* reach reproductive maturity within 2 years, and both are broadcast spawners, releasing eggs, and sperm into the water column (Beasley, Dardeau, & Schroeder, 2003; Gotelli, 1991).
The larvae in *L. virgulata* can spend 3–20 days in the water column before settlement (Gotelli, 1991). The duration of the larval stage for *L. hebes* is unknown. Both *L. hebes* and *L. virgulata* have been successful at colonizing artificial structures in the Gulf of Mexico, including jetties within the subtidal zone (Williamson et al., 2011). A strong holdfast and a rigid, yet flexible skeleton, allows these two species to colonize habitats with swift currents and wave action such as that found in jetties (Williamson et al., 2011). These life history characteristics of *L. hebes* and *L. virgulata* include relatively fast maturation, broadcast spawning, long survival of larval stages, and adaptations for successful establishment in high energy environments, demonstrate the high potential for dispersal and colonization of new regions of these species.

Old, incomplete, or damaged records in addition to a lack of easily identifiable morphological traits among species make gorgonians particularly difficult to classify (Sánchez, 2007). For example, the genus *Leptogorgia* was initially split into two genera—*Leptogorgia* and *Lophogorgia*—by Milne-Edwards and Haime (1857). Species in the *Leptogorgia* genus are described as having disk-spindles in the outer coenenchyme, while *Lophogorgia* species have spindles more closely resembling flat rods and were described mostly in the Eastern Pacific, Western Atlantic, Caribbean, and along the eastern and southern coasts of Africa (Bayer, 1961). However, in 1988 these morphological distinctions were questioned and the two genera were united into one as *Leptogorgia* (Grashoff, 1988). In 2017, Poliseno et al. conducted a phylogenetic study of *Leptogorgia*, using specimens from a wide geographical area, including the eastern and Western Atlantic, the Eastern Pacific, and the Mediterranean. They reconstructed two phylogenies, one based on complete mitochondrial genomes and the other based on a partial fragment of the mitochondrial MutS gene (*mtMutS*). While their phylogeny based on complete mitochondrial genomes only has eleven species of the family Gorgoniidae, including six species of *Leptogorgia*, the one based on the single *mtMutS* gene includes 109 species, providing greater taxonomic resolution. In their study, Poliseno et al. (2017) also estimate divergence times with a fossil calibration point of 28.4 Ma based on the stratigraphy and dating of the presence of substitution saturation and long branch attraction (i.e., Kitahara et al., 2014), it is only an issue within the Hexacorallia and it does not affect the Octocorallia, such as the gorgonian corals in our study (Figueroa & Baco, 2015). Complete mitochondrial genomes have been demonstrated to provide robust and well-supported phylogenies for Octocorallia (e.g., Figueroa & Baco, 2014; Figueroa & Baco, 2015; Kayal, Roure, Philippe, Collins, & Lavrov, 2013; Poliseno et al., 2017), while the use of single mitochondrial genes has been demonstrated to result in incongruent largely unresolved trees across a wide range of taxa (Havird & Santos, 2014; Knaus, Cronn, Liston, Pilgrim, & Schwartz, 2011; Luo et al., 2011; Nadimi, Daubois, & Hijri, 2016; Pacheco et al., 2011; Rohland et al., 2007; Urantowka, Kroczak, & Mackiewicz, 2017; Wang et al., 2017; Willerslev et al., 2009). Therefore, we expect that the taxonomic position of *L. hebes* and *L. virgulata* weakly supported. Therefore, in our study, we analyze both complete mitochondrial genomes and the *mtMutS* gene.

Our study focuses on two species of *Leptogorgia* from the Gulf of Mexico, *L. hebes* and *L. virgulata*. We have two main goals. The first is to determine the taxonomic position of *L. hebes* (formerly classified in the genus *Lophogorgia* by Bayer, 1961) and of *L. virgulata*. The analyses by Poliseno et al. (2017) did not include complete mitochondrial genomes for these two species and their phylogeny based on the partial *mtMutS* gene leaves the phylogenetic position of both *L. hebes* and *L. virgulata* weakly supported. Therefore, in our study, we analyze both complete mitochondrial genomes and the *mtMutS* gene. Although mitochondrial genomes have been shown to be problematic for phylogenetic reconstruction of scleractinian corals due to the presence of substitution saturation and long branch attraction (i.e., Kitahara et al., 2014), it is only an issue within the Hexacorallia and it does not affect the Octocorallia, such as the gorgonian corals in our study (Figueroa & Baco, 2015). Complete mitochondrial genomes have been demonstrated to provide robust and well-supported phylogenies for Octocorallia (e.g., Figueroa & Baco, 2014; Figueroa & Baco, 2015; Kayal, Roure, Philippe, Collins, & Lavrov, 2013; Poliseno et al., 2017), while the use of single mitochondrial genes has been demonstrated to result in incongruent largely unresolved trees across a wide range of taxa (Havird & Santos, 2014; Knaus, Cronn, Liston, Pilgrim, & Schwartz, 2011; Luo et al., 2011; Nadimi, Daubois, & Hijri, 2016; Pacheco et al., 2011; Rohland et al., 2007; Urantowka, Kroczak, & Mackiewicz, 2017; Wang et al., 2017; Willerslev et al., 2009). Therefore, we expect that the taxonomic position of *L. hebes* and *L. virgulata* will be fully resolved by reconstructing their phylogeny using mitochondrial genomes.

Our second goal is to estimate divergence times of Eastern Pacific and Western Atlantic *Leptogorgia* species. Since previous research has shown that fossil-calibrated phylogenetic reconstruction based on single mitochondrial genes results in an overestimation of divergence times (Duchêne, Archer, Vilstrup, Caballero, & Morin, 2011; McCormack, Heled, Delaney, Peterson, & Knowles, 2011), we will base our estimates of diversification times between Eastern Pacific and Western Atlantic lineages of *Leptogorgia* to targeting complete mitochondrial genomes. We reconstruct a fossil-calibrated phylogenetic tree for *Leptogorgia* species based on complete mitochondrial genomes and using *Eunicella* as an outgroup. We use a fossil calibration point of 28.4 Ma based on the stratigraphy and dating of the Red Bluff Formation in Mississippi where the oldest fossils of *Eunicella* have been recovered (Cushing, Boswell, & Hosman, 1964; Demchuk & Gary, 2009; Kocurko & Kocurko, 1992; Prothero, Ivany, & Nesbitt, 2003; Tew, 1992). Among Octocorallia, skeletal diversity, such as morphology of sclerites, is a key character for taxonomic identification (Goffredo & Dubinsky, 2016). Sclerites with a balloon club shape are a distinguishing characteristic that is unique to the genus *Eunicella* (Goffredo & Dubinsky, 2016; Kocurko & Kocurko, 2016).
Fossil sclerites with balloon club shape have been found in the Red Bluff Formation in Mississippi and have been clearly attributed to *Eunicella* (Kocurko & Kocurko, 1992). Stratigraphy of the Red Bluff Formation and dating of this layer within the Oligocene (23–34 Ma) has been intensely studied (i.e., Cushing et al., 1964; Demchuk & Gary, 2009; Hosman, 1996; Prothero et al., 2003; Tew, 1992).

The timeline proposed by Poliseno et al. (2017) for the divergence between Eastern Pacific and Western Atlantic *Leptogorgia* species coincides with evidence that a land bridge between North and South America began to emerge between 23 and 25 Ma when the Panama Arc collided with South America (Bacon et al., 2015). However, despite this initial emergence and given the life history characteristics of shallow water *Leptogorgia* species such as *L. hebes* and *L. virgulata* that enhance dispersal and colonization (Beasley & Dardeau, 2003; Cairns & Bayer, 2009; Gotelli, 1988, 1991; Williamson et al., 2011), gene flow is likely to have continued between the Western Atlantic and Eastern Pacific until full closure of the Central American Seaway (Bacon et al., 2015; Cowman & Bellwood, 2013; Lessios, 2008; Thacker, 2017). Therefore, we hypothesize that the divergence times of Eastern Pacific and Western Atlantic *Leptogorgia* lineages to be younger than previously suggested (Poliseno et al., 2017) with the majority of speciation events occurring after 10 Ma when significant seawater exchange between the Pacific and Atlantic Ocean ceased (i.e., Bacon et al., 2015; Montes et al., 2015; O’Dea et al., 2016).

### 2 | MATERIALS AND METHODS

#### 2.1 | Study sites and sample collection

Six sites in the Gulf of Mexico off the coast of the United States in South Padre Island, Texas, were sampled for 24 *Leptogorgia* specimens (seven *L. hebes* and 17 *L. virgulata*) by divers collecting coral fragments between June 2014 and July 2017 (Table 1). Once collected, samples were preserved in ethanol and stored at 0°C. Voucher specimens are deposited and curated at the University of Texas Rio Grande Valley’s Coastal Studies Laboratory and are available upon request under GenBank accession numbers MK0301586–MK0301592 for specimens of *L. virgulata* and MN052675–MN052677 for specimens of *L. hebes*.

#### Table 1. *Leptogorgia* sp. found at all sites and site types with respective dates, coordinates, and depths

| Location | Date       | Lat     | Lon     | Depth (m) | # of samples/species collected |
|----------|------------|---------|---------|-----------|-------------------------------|
| Port Isabel Reef | 6/3/2014 | 25.9684 | -97.0669 | 22        | 1 *L. hebes*; 1 *L. virgulata* |
| Port Mansfield Liberty Ship | 12/12/2014 | 26.4296 | -97.0241 | 24        | 1 *L. hebes*; 1 *L. virgulata* |
| Jack up Rigs/East Bank | 7/7/2016 | 26.1021 | -96.9377 | 32        | 1 *L. virgulata* |
| Port Mansfield Liberty Ship | 9/2/2016 | 26.4296 | -97.0241 | 24        | 1 *L. virgulata* |
| Port Isabel Reef | 9/15/2016 | 25.9684 | -97.0669 | 22        | 1 *L. hebes*; 1 *L. virgulata* |
| Port Mansfield Liberty Ship | 3/20/2017 | 26.4296 | -97.0241 | 24        | 1 *L. hebes* |
| MU 726 A | 6/8/2017 | 27.8146 | -96.7622 | 24        | 4 *L. virgulata* |
| Texas Clipper | 7/8/2017 | 26.1903 | -96.8614 | 15-41     | 2 *L. virgulata* |
| SPI Jetty | 7/30/2017 | 26.0674 | -97.1504 | 5         | 3 *L. hebes*; 6 *L. virgulata* |

Three to five individual polyps were picked off from each coral sample, depending on the size and quality of preservation of the coral fragment. Polyps were visually inspected under a stereo microscope and picked off the coral stalk using forceps. Forceps were sterilized in between each sample using 100% bleach and 100% ethanol. If individual polyps were difficult to distinguish, an ~0.5 cm long piece was broken off of the coral fragment. The PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific) was used to extract DNA from each sample following the manufacturer’s standard protocol. Prior to extraction, coral polyps were rehydrated for 1–2 hr in molecular grade water and then digested for at least 5 hr. The final DNA product was eluted two times for maximum yield. The elution buffer was heated to 55°C prior to use, and 60 µl of were used for both elutions. The concentration of the extracted DNA was measured using a Qubit fluorometer (Life Technologies Inc.).

Polymerase Chain Reaction (PCR) amplification was performed on 0.1–5.0 ng template DNA from 24 samples in order to target the mtMutS gene with forward primer ND42599F (GCCATTATGGTTAACTATTAC; France & Hoover, 2002) and reverse primer Mut3458R (TSGAGCAAAAGCCACTCC; Sanchez, McFadden, France, & Lasker, 2003). The PCR mix consisted of the following in 25 µl total volume: 16.05 µl nuclease free water, 2.5
TABLE 2  All 182 Leptogorgia mtMutS sequences incorporated into the mtMutS phylogeny with corresponding GenBank accession numbers

| Species               | Accession # | Species               | Accession # | Species               | Accession # | Species               | Accession # |
|-----------------------|-------------|-----------------------|-------------|-----------------------|-------------|-----------------------|-------------|
| Leptogorgia virgulata | MN159153    | Leptogorgia alba      | KX721205    | Leptogorgia obscura    | KX721210    | Leptogorgia taboguilla | LT221102    |
| Leptogorgia virgulata | MN159154    | Leptogorgia alba      | KX721203    | Leptogorgia pictcola   | KY683795    | Leptogorgia tricora    | LT221111    |
| Leptogorgia virgulata | MN159155    | Leptogorgia alba      | KX721202    | Leptogorgia pulcherrima | KY683794    | Leptogorgia viridula   | LT221109    |
| Leptogorgia virgulata | MN159156    | Leptogorgia alba      | KX721201    | Leptogorgia pulcherrima | KY683793    | Leptogorgia viridula   | KY683796    |
| Leptogorgia virgulata | MN159157    | Leptogorgia alba      | KX721195    | Leptogorgia pulcherrima | KY683793    | Leptogorgia viridula   | KY683796    |
| Leptogorgia virgulata | MN159158    | Leptogorgia alba      | KY559410    | Leptogorgia pulcherrima | KY68443    | Leptogorgia violacea   | KY68448     |
| Leptogorgia virgulata | MN159159    | Leptogorgia barnardi  | KY767314    | Leptogorgia pulmila    | KY767312    | Leptogorgia violette   | KY68446     |
| Leptogorgia virgulata | MN159160    | Leptogorgia alba      | HG170363    | Leptogorgia pulmila    | LT221116    | Leptogorgia virgulata  | KY68458     |
| Leptogorgia virgulata | MN159161    | Leptogorgia alba      | HG170357    | Leptogorgia pulmila    | KY68449    | Leptogorgia virgulata  | KY68458     |
| Leptogorgia virgulata | MN159162    | Leptogorgia alba      | HG170304    | Leptogorgia ramulus    | KY68451    | Pacifigorgia bayeri    | HG917044    |
| Leptogorgia virgulata | MN159163    | Leptogorgia alba      | AY268452    | Leptogorgia ramulus    | KX767322    | Pacifigorgia cairns    | KY59409     |
| Leptogorgia virgulata | MN159164    | Leptogorgia alba      | LT221108    | Leptogorgia regis      | LT221101    | Pacifigorgia cairns    | KY68451     |
| Leptogorgia virgulata | MN159165    | Leptogorgia alba      | LT221113    | Leptogorgia regis      | LT221100    | Pacifigorgia cairns    | KY68451     |
| Leptogorgia virgulata | MN159166    | Leptogorgia barnardi  | KY236043    | Leptogorgia regis      | LT221099    | Pacifigorgia cairns    | KY68451     |
| Leptogorgia virgulata | MN159167    | Leptogorgia barnardi  | KY553145    | Leptogorgia regis      | LT221098    | Pacifigorgia cairns    | KY68451     |
| Leptogorgia virgulata | MN159168    | Leptogorgia cf.palmata| KY559406    | Leptogorgia rigida     | GQ342496    | Pacifigorgia exilis    | KX351871    |
| Leptogorgia virgulata | MN159169    | Leptogorgia cf.palmata| KY236042    | Leptogorgia rubra      | KX767323    | Pacifigorgia firma     | KX351872    |
| Leptogorgia hebes     | MN159170    | Leptogorgia cf.palmata| KY236030    | Leptogorgia sarmentosa | KY559411    | Pacifigorgia firma     | KX351872    |
| Leptogorgia hebes     | MN159171    | Leptogorgia cf.palmata| KY236031    | Leptogorgia sp.        | KX767315    | Pacifigorgia irene     | KX351873    |
| Leptogorgia hebes     | MN159172    | Leptogorgia cf.palmata| AY268460    | Leptogorgia sp.        | KX721204    | Pacifigorgia irene     | KX687024    |
| Leptogorgia hebes     | MN159173    | Leptogorgia chilensis | JN866554    | Leptogorgia sp.        | KX59412     | Pacifigorgia macalilla | KX351876    |
| Leptogorgia hebes     | MN159174    | Leptogorgia chilensis | HG170373    | Leptogorgia sp.        | KX236033    | Pacifigorgia macalilla | KX351874    |
| Leptogorgia hebes     | MN159175    | Leptogorgia chilensis | HG170374    | Leptogorgia sp.        | KX236032    | Pacifigorgia macalilla | KX351875    |
| Leptogorgia hebes     | MN159176    | Leptogorgia chilensis | HG170375    | Leptogorgia sp.        | KX683791    | Pacifigorgia medio     | GQ342497    |
| Acanthogorgia sp     | AY268461    | Leptogorgia cf.palmata| HG170385    | Leptogorgia sp.        | LT221114    | Pacifigorgia rubicunda | HG917027    |
| Antillogorgia acerosa | JX152763    | Leptogorgia cortes    | LT221105    | Leptogorgia sp.        | LT221115    | Pacifigorgia sculpta   | KX351877    |
| Antillogorgia sp. 1   | JX152764    | Leptogorgia cepidata  | KX767318    | Leptogorgia sp.        | LT221106    | Pacifigorgia senta     | LT221107    |
| Antillogorgia sp. 2   | JX152765    | Leptogorgia cepidata  | AY268450    | Leptogorgia sp.        | KX236035    | Pacifigorgia smithsoniana | HG917023    |
| Eugorgia ampla       | KX767336    | Leptogorgia cepidata  | HG17047     | Leptogorgia sp.        | KX236034    | Pacifigorgia stenobrochis | HG917018    |
| Eugorgia daniana     | HG917048    | Leptogorgia cepidata  | AY268445    | Leptogorgia sp.        | KX236037    | Pacifigorgia stenobrochis | AY166240    |
| Eugorgia daniana     | LT221110    | Leptogorgia cepidata  | KX767319    | Leptogorgia sp.        | KX236036    | Phyllogorgia dilata     | AY166248    |
| Eugorgia multifida   | GQ342494    | Leptogorgia cepidata  | KX721209    | Leptogorgia sp.        | KX236039    | Pseudopterogorgia acerosa | AY166241    |
| Eugorgia mutabilis   | KY559405    | Leptogorgia cepidata  | KX767326    | Leptogorgia sp.        | KX236038    | Pseudopterogorgia americana | AY166243    |
| Eugorgia mutabilis   | LT221112    | Leptogorgia cepidata  | KX767329    | Leptogorgia sp.        | KX236041    | Pseudopterogorgia australiensis | AY166242    |
TABLE 2

| Species Accession # | Species Accession # | Species Accession # | Species Accession # |
|-------------------|-------------------|-------------------|-------------------|
| KY236040          | JN866557          | Leptogorgia flexilis | b KX767328        |
| Eugorgia rubens    | Pseudopterogorgia elisabethae | Leptogorgia sp. | b KX767327 |
|                    |                   |                   |                   |
| Eugorgia siedenburgae | Leptogorgia flexilis | b KX767325           |                   |
|                    |                   |                   |                   |
| Leptogorgia flexilis | b KX767324           |                   |                   |
| Eunicella cavolinii | Leptogorgia flexilis | b KX767323           |                   |
|                    |                   |                   |                   |
| Leptogorgia flexilis | b KX767322           |                   |                   |
| Eunicella albicans  | Leptogorgia flexilis | b KX767321           |                   |
|                    |                   |                   |                   |
| Leptogorgia flexilis | b KX767320           |                   |                   |
| Eunicella cavolinii | Leptogorgia flexilis | b KX767319           |                   |
|                    |                   |                   |                   |
| Leptogorgia flexilis | b KX767318           |                   |                   |
| Eunicella albicans  | Leptogorgia flexilis | b KX767317           |                   |
|                    |                   |                   |                   |
| Leptogorgia flexilis | b KX767316           |                   |                   |
| Eunicella cavolinii | Leptogorgia flexilis | b KX767315           |                   |
|                    |                   |                   |                   |
| Leptogorgia flexilis | b KX767314           |                   |                   |
| Eunicella cavolinii | Leptogorgia flexilis | b KX767313           |                   |
|                    |                   |                   |                   |

Sequences from this study.
New sequences from GenBank.
Sequences used by Poliseno et al. (2017).

2.3 | Sequence assembly and alignment

For each specimen, the sequences for the forward and reverse strands were assembled with the software CLC Workbench 7.9.1 (CLC Bio) using the settings: minimum aligned read length = 500 bp, alignment stringency = high, conflicts = ambiguity nucleotides, trim sequence ends and trim using quality scores limit = 0.05. A cutoff was used were only bases with Phred scores of 20 or more were kept. A consensus sequence was generated from each assembly. Qiagen's CLC Workbench 7.9.1 was used to align the mtMutS sequences. The mtMutS sequences were aligned using Qiagen's CLC Main Workbench 7 software and include 24 sequences from this study, the 114 sequences examined in Poliseno et al. (2017) and 43 novel sequences available in GenBank for a total of 182 sequences (Table 2). The alignment was visually inspected for errors and inconsistencies. The final mtMutS alignment was 766 bp in length.

The Illumina sequence reads were assembled using the software CLC Genomics Workbench 11. Default settings were used with reads mapped back to contigs (mismatch cost = 2, insertion cost = 3, deletion cost = 3, length fraction = 0.5, similarity fraction = 0.8). The sequences obtained from the assemblies included the full mitochondrial genome for each specimen with an average read coverage of over 100 and a minimum coverage of 35. The assembled
TABLE 3 All 21 gorgonian mitochondrial genomes and their corresponding GenBank accession number

| Species                     | Size (bp) | GenBank Accession # |
|-----------------------------|-----------|---------------------|
| Leptogorgia virgulata       | 18,845    | MK301586            |
| Leptogorgia virgulata       | 18,845    | MK301587            |
| Leptogorgia virgulata       | 18,824    | MK301588            |
| Leptogorgia virgulata       | 18,845    | MK301589            |
| Leptogorgia virgulata       | 18,824    | MK301591            |
| Leptogorgia virgulata       | 18,845    | MK301592            |
| Leptogorgia hebes           | 19,247    | MN052675            |
| Leptogorgia hebes           | 19,247    | MN052676            |
| Leptogorgia hebes           | 19,247    | MN052677            |
| Pseudopterogorgia bipinnata | 18,733    | DQ640646            |
| Leptogorgia capverdensis     | 18,722    | KY553145            |
| Leptogorgia gaini           | 19,682    | KY559404            |
| Eugorgia mutabilis          | 19,157    | KY559405            |
| Leptogorgia cf. palma       | 18,731    | KY559406            |
| Eunicella albicans          | 19,175    | KY559407            |
| Eunicella cavolinii         | 19,316    | KY559408            |
| Pacificigorgia caimsi       | 19,156    | KY559409            |
| Leptogorgia alba            | 18,848    | KY559410            |
| Leptogorgia sarmentosa      | 18,722    | KY559411            |
| Leptogorgia sp.             | 18,849    | KY559412            |

*The 10 novel mt genomes sequenced in this study.

TABLE 4 Data block definitions for partition analysis

| Region | Codon positions |
|--------|-----------------|
|        | 1      | 2      | 3      |
| (A)    | 1–708  | 2–708  | 3–708  |
| Atp6   | 709–924| 710–924| 711–924|
| Cox1   | 925–2,550| 926–2,550| 927–2,550|
| Cox2   | 2,551–3,312| 2,552–3,312| 2,553–3,312|
| Cox3   | 3,313–4,098| 3,314–4,098| 3,315–4,098|
| Cytb   | 4,099–5,273| 4,100–5,273| 4,101–5,273|
| MutS   | 5,274–8,231| 5,275–8,231| 5,276–8,231|
| Nad1   | 8,232–9,203| 8,233–9,203| 8,234–9,203|
| Nad2   | 9,204–10,361| 9,205–10,361| 9,206–10,361|
| Nad3   | 10,362–10,734| 10,363–10,734| 10,364–10,734|
| Nad4   | 10,735–12,183| 10,736–12,183| 10,737–12,183|
| Nad4L  | 12,184–12,477| 12,185–12,477| 12,186–12,477|
| Nad5   | 12,478–14,320| 12,479–14,320| 12,480–14,320|
| Nad6   | 14,321–14,878| 14,322–14,878| 14,323–14,878|
| rRNA (12s) | 14,879–15,807 | 14,879–15,807 |
| rRNA (16s) | 15,808–17,999 | 15,808–17,999 |

(B) MutS 1–766 2–766 3–766

Note: (A) Mitochondrial genome concatenated alignment including 14 protein-coding genes and 2 RNAs. (B) mtMutS alignment.

2.4 Phylogenetic analyses

Both mtMutS and complete mt genome alignments were used in phylogenetic analyses using maximum likelihood (ML) and Bayesian methods. The model of evolution and partitioning scheme was determined by PartitionFinder v1.1.1 (Lanfear, Calcott, Kainer, Mayer, & Stamatakis, 2014) using linked branches and the Akaike information criterion (AIC). The RAxML v8.0.0 program (Stamatakis, 2017) was used to conduct the ML analyses and Mr. Bayes 3.1 (Ronquist & Huelsenbeck, 2003) was used for the Bayesian analyses. Data blocks were created for mtMutS based on codon position (Table 4). PartitionFinder selected GTR + G as the best evolutionary model for three partitions: (a) mtMutS1; (b) mtMutS2; and (c) mtMutS3.

For the mitochondrial genome analyses, data blocks were created based on codon positions for all 14 protein-coding genes (Cox1, Nad1, CytB, Nad2, Nad3, Nad4L, mtMutS, Nad2, Nad5, Nad4, Cox3, Atp6, Atp8, and Cox2) and two ribosomal RNAs (Table 4). For the ML analysis, PartitionFinder selected General Time Reversible plus Gamma (GTR + G) as the best evolutionary model for 11 partition subsets and GTR + I+G for three subsets (Table 5). For the Bayesian analysis, the data were partitioned into 16 subsets. PartitionFinder selected GTR + I as the best model for two subsets, F81 for one subset, GTR + G for four subsets, GTR + I+G for two subsets, GTR for one subset, HKY for two subsets, HKY + G for two subsets, and HKY + I+G for one subset (Table 5).

The best maximum likelihood tree was reconstructed with RAxML for both, the mtMutS alignment and the mt genome alignment, using bootstrap values from 10,000 replicates. Note that in RAxML partitions cannot be analyzed with different evolutionary models and one model must be used for all partitions. Therefore, the mt genome alignment was analyzed under a GTR + G model given that PartitionFinder selected this as the best model for 11 of the 14 partitions. Phylogenetic trees were also reconstructed for both by Bayesian methods. Using Mr. Bayes, four chains were carried out for 1,100,000 generations, sampling every 200th generation. After inspecting the trace files generated by the Bayesian Markov Chain Monte Carlo (MCMC) runs, the initial 100,000 of sampled generations were omitted prior to building the consensus
An uncorrelated log-normal relaxed clock model was used along with the calibrated yule speciation model. The tree was calibrated based on the earliest fossil evidence for Eunicella (Kocurko & Kocurko, 1992) with a date of origination set to 28.4 Ma (mean = 1 and standard deviation = 1). One chain was carried out for 10,000,000 generations, sampling every 1,000th generation. After inspecting the trace files generated by the Bayesian Markov Chain Monte Carlo (MCMC) runs, the initial 25% of sampled generations were omitted prior to building the tree. Mean divergence times were summarized with TreeAnnotator.

### 3 | RESULTS

#### 3.1 | Mitochondrial MutS phylogeny

The 17 sequences of mtMutS of L. virgulata are identical, while the seven sequences of L. hebes range from 99.74% to 100% identity. The phylogenetic reconstruction based on mtMutS included the 17 sequences of L. virgulata and the seven sequences of L. hebes generated by this study (Figure 1). These 24 sequences were combined with 158 additional Leptogorgia mtMutS sequences and two of Eunicella (outgroup), downloaded from GenBank. The topology between the Bayesian and maximum likelihood analyses is relatively similar. There are nine major clades (A–I) that are strongly supported (>70 bootstrap and >95 posterior probability) except for Clade F (<50 bootstrap and <50 posterior probability), each corresponding to taxa from a particular geographic region (Figure 1).

Clade A corresponds to species found in the Eastern Atlantic and Mediterranean. Clade A is sister to Clade B, which corresponds to species found in South Africa. Clade A and B, along with Pseudopterogorgia fredericki and Pseudopterogorgia australiensis form a clade that is weakly supported (51 bootstrap and 85 posterior probability) and sister to all other species (Figure 1). This sister clade with the remaining species is moderately supported (77 bootstrap and 86 posterior probability) and contains clades C–I.

Clade C (100 bootstrap and posterior probability) consists of 11 different species representing multiple genera (Pseudopterogorgia, Antillogorgia, Gorgonia, and Phyllogorgia). All species in Clade C are found in the Caribbean (Figure 1). Clade C is sister to the remaining species which form a strongly supported group (97 bootstrap and 86 posterior probability) containing clades D–I. Clade D (100 bootstrap and posterior probability) is made up of species of Pacificigorgia along with a few species of Leptogorgia, all of which are from the Eastern Pacific (Figure 1). Clade D is sister to a strongly supported clade (100 bootstrap and 100 posterior probability) that consist of the remaining species within clades E–I. Clade E (89 bootstrap and 100 posterior probability) consists of several species of Leptogorgia and Eugorgia all from the Eastern Pacific (Figure 1). Clade E is sister to a strongly supported group (91 bootstrap and 100 posterior probability) containing clades F–I. Clade F does not have statistical support (<50 bootstrap and <50 posterior probability). Within clade F are Leptogorgia violacea, L. punicea, and L. rubra along with a well-supported clade (90 bootstrap and 99 posterior probability).

### Table 5: Partition scheme for the concatenated mitochondrial genome alignment for ML and Bayesian analyses

| Subset | Best model | # of Sites | Maximum likelihood partitions |
|--------|------------|------------|-----------------------------|
| 1      | GTR + G    | 808        | NAD2, ATP6, NAD6            |
| 2      | GTR + I+G  | 2,333      | NAD6, NAD4, CYTb, NAD5, ATP6, NAD4L, NAD12 |
| 3      | GTR + G    | 1,423      | CYTb2, CYTb3, ATP6          |
| 4      | GTR + I+G  | 3,193      | ATP8, 16S rRNA, 12S rRNA    |
| 5      | GTR + G    | 582        | NAD2, ATP8, NAD3            |
| 6      | GTR + G    | 782        | NAD2, NAD1, ATP3            |
| 7      | GTR + G    | 1,605      | CYTb2, NAD4L, NAD3, CYTb3, NAD1, NAD1 |
| 8      | GTR + G    | 1,058      | CYTb2, CYTb3, CYT1          |
| 9      | GTR + G    | 546        | NAD6, NAD4L, CYT3           |
| 10     | GTR + I+G  | 1,490      | NAD5, CYTb, NAD4            |
| 11     | GTR + G    | 1,110      | MUT1, NAD3                  |
| 12     | GTR + G    | 986        | MUT5                        |
| 13     | GTR + G    | 1,600      | NAD5, MUT5                  |
| 14     | GTR + G    | 483        | NAD4                        |

| Subset | Best model | # of Sites | Bayesian partitions |
|--------|------------|------------|---------------------|
| 1      | GTR + I    | 808        | NAD6, ATP6, NAD2    |
| 2      | F81        | 720        | NAD2, NAD4L, ATP6   |
| 3      | GTR + G    | 1,788      | ATP8, CYT1, CYTb2, ATP6, NAD1, NAD4L, CYT3 |
| 4      | HKY + I+G  | 3,193      | ATP8, 16S rRNA, 12S rRNA |
| 5      | HKY        | 196        | NAD3, ATP8          |
| 6      | GTR + I    | 1,351      | CYT1, NAD1, CYTb3, NAD4L, NAD3 |
| 7      | GTR + I    | 1,382      | CYT1, NAD2, CYTb3, CYT2 |
| 8      | HKY        | 254        | CYT2                |
| 9      | GTR + I+G  | 1,490      | CYTb1, NAD4, NAD1   |
| 10     | GTR + I+G  | 1,675      | CYTb2, NAD5, NAD4, NAD6 |
| 11     | HKY + G    | 1,563      | NAD6, MUT5, CYTb3   |
| 12     | HKY + G    | 1,110      | MUT5, NAD3          |
| 13     | GTR + G    | 986        | MUT5                |
| 14     | GTR        | 386        | NAD2                |
| 15     | GTR + G    | 483        | NAD4                |
| 16     | GTR + G    | 614        | NAD5                |

Note: Superscript numbers indicate codon position 1, 2, or 3.
containing all specimens of *L. hebes* (Figure 1). All species in clade F are from the Western Atlantic and Gulf of Mexico. Clade F is sister to a clade with no statistical support (<50 bootstrap and <50 posterior probability) containing the remaining species within clades G-I. Clade G (100 bootstrap and posterior probability) contains several species of *Eugorgia* along with *Leptogorgia pumila*, all from the Eastern Pacific (Figure 1). Clade G is sister to a group that is not statistically supported (<50 bootstrap and <50 posterior probability) and contains clades H and I. Clade H (100 bootstrap and posterior probability) consists of species from the Western Atlantic and Gulf of Mexico, *Leptogorgia gracilis* and *L. virgulata* (Figure 1). Specimens of *L. virgulata* form a clade that is moderately supported in the ML tree (64 bootstrap) and strongly supported in the Bayesian tree (100 posterior probability). This *L. virgulata* clade is sister to *L. gracilis*. Clade H is sister to Clade I (100 bootstrap and posterior probability) which contains numerous species of *Leptogorgia* from the Eastern Pacific (Figure 1).

### 3.2 Mitochondrial genomes

A total of ten new *Leptogorgia* mitochondrial genomes were obtained—seven *L. virgulata* mt genomes and three *L. hebes* mt genomes. The *L. virgulata* mt genomes range in length from 18,824 to 18,845, and all *L. hebes* mt genomes are 19,247 bp. The *L. virgulata* mt genomes range from 99.87% to 100% identity while those for *L. hebes* range
from 99.5% to 99.98% identity. All ten mt genomes consist of 14 protein-coding genes (Cox1, Nad1, CytB, Nad6, Nad3, Nad4L, mtMutS, Nad2, Nad5, Nad4, Cox3, Atp6, Atp8, and Cox2, in respective order) and two ribosomal RNAs (Figure 2). Both species have what is presumed to be the ancestral gene order found in octocorals (Brugler & France, 2008; Figueroa & Baco, 2014, 2015; Medina, Collins, Takaoka, Kuehl, & Boore, 2006; Park et al., 2012; Uda et al., 2011).

3.3 Mitogenomic phylogeny

The phylogenetic reconstruction based on full mitochondrial genomes included 7 mt genomes of L. virgulata and 3 mt genomes of L. hebes generated by this study. These mt genomes were combined with 11 additional mt genomes from the family Gorgoniidae and two mt genomes of Eunicella (outgroup), downloaded from GenBank (Table 3). Maximum likelihood and Bayesian analyses resulted in similar topology (Figure 3). There are nine well-supported clades (clades I–IX) that roughly match those identified in the mtMuS phylogeny (Figure 3). Clade I (96 bootstrap and 100 posterior probability) is made up of Leptogorgia palma (mtMuS clade B) as sister to clade II (100 bootstrap and posterior probability, mtMuS clade A) which contains Leptogorgia capverdensis and Leptogorgia sarmientosa. Clade I is sister to all other Leptogorgia, but this sister clade is weakly supported (61 bootstrap and 85 posterior probability) and contains Pseudopterogorgia bipinnata (mtMuS clade C) as sister to clade III. Clade III (100 bootstrap and posterior probability) contains Pacifigorgia cairnsi (mtMuS clade D) as sister to clade IV (100 bootstrap and posterior probability). Clade IV contains Leptogorgia sp. (KY559412) as sister to clade V (89 bootstrap and 100 posterior probability). Clade V consists of clade VI (90 bootstrap and 100 posterior probability) as sister to clade VII (65 bootstrap and 100 posterior probability). Clade VI has Leptogorgia alba (mtMuS clade I) as sister to Eugorgia mutabilis (mtMuS clade E). Clade VII contains clade VIII (mtMuS clade F) as sister to clade IX (mtMuS clade H). Clade VIII (100 bootstrap and 100 posterior probability) consists of seven specimens of L. hebes. Clade IX (100 bootstrap and posterior probability) consists of seven specimens of L. virgulata. Within the L. hebes clade, two individuals (accession #s MN052676 and MN052675) form a strongly supported clade (97 bootstrap and 81 posterior probability). The L. virgulata clade also has two individuals (accession #s MK0301589 and MK0301591) forming an internal clade, strongly supported by maximum likelihood only (96 bootstrap).

3.4 Mitogenomic divergence time estimation

The phylogenetic reconstruction based on mitochondrial genomes using fossil-calibrated coalescent methods as implemented by Bayesian analyses in BEAST (Figure 4) resulted in topology similar to the maximum likelihood (ML) and Bayesian analysis with RaxML and Mr. Bayes. Emerging from the root are two main clades which diverged from one another 25.96 Ma. Within the first main
clade, there are two branches containing a single species each—*P. bipinnata* and *Leptogorgia cf. palma* which diverged 25.01 and 21.95 Ma, respectively. Following these two branches is a branch containing *Leptogorgia gaini*, which diverged 12.75 Ma from a sister subclade consisting of *L. sarmentosa* and *L. capverdensis*. *Leptogorgia sarmentosa* and *L. capverdensis* diverged from one another 0.46 Ma. However, this subclade is weakly supported. The grouping of the five aforementioned species is consistent between all three mitogenomic trees, with the exception of *P. bipinnata*. On the ML and Mr. Bayes’ Bayesian trees, *P. bipinnata* does not emerge until after the 4 other species—*L. cf. palma*, *L. gaini*, *L. sarmentosa*, and *L. capverdensis*—and it forms a basal branch.

In the second main clade, *P. cairnsi* diverges at 20.38 Ma and forms a basal branch to a subclade containing *L. hebes*, *E. mutabilis*, *Leptogorgia sp.* (KY559412), *L. alba*, and *L. virgulata*. *Leptogorgia hebes* is the first species to diverge from this subclade at 10.89 Ma. Following the *L. hebes* group are two branches containing *E. mutabilis* and *Leptogorgia sp.* (KY559412), diverging at 9.88 and 6.75 Ma, respectively. *Leptogorgia alba* and *L. virgulata* then diverged from one another at 5.82 Ma. In the ML and Mr. Bayes’ Bayesian trees, *E. mutabilis* and *L. alba* are sister to one another, but on the BEAST tree *L. alba* is sister to *L. virgulata*.

4 | DISCUSSION
4.1 | Mitochondrial MutS phylogeny

The reconstructed *mtMutS* phylogeny uses 68 new *mtMutS* sequences (24 from this study and 44 from GenBank) added to the sequences used in the phylogenetic tree by Poliseno et al. (2017). This new *mtMutS* phylogeny agrees with the phylogeny presented by Poliseno et al. (2017). The *Leptogorgia* species from South Africa form a sister clade to species from the Eastern Atlantic and Mediterranean
(Figure 1). The Caribbean clade from Poliseno et al. (2017) is also recovered (clade C, Figure 1). There are several clades with species exclusively from the Eastern Pacific. Most notably, Eastern Pacific clade I is sister to the Western Atlantic and Gulf of Mexico clade H that contains *L. virgulata* and *L. gracilis*. As in Poliseno et al. (2017), the major clades identified (A–I) have species that are exclusive to a particular geographic region. And while all of these clades are strongly supported (except for clade F), relationships between several of these clades is not clear due to low or no statistical support. The South African clade (clade B, Figure 1) contains *L. palma*, formerly known as *Lophogorgia crista*, which is the type species for the *Lophogorgia* genus (Poliseno et al., 2017). Because this South African group is monophyletic and strongly supported, Poliseno et al. (2017) recommend that the genus *Lophogorgia* be resurrected and assigned to this clade. This complicates matters when it comes to other species formerly classified as *Lophogorgia* by Bayer (1961) which are not in the South African clade-specifically, *L. dichotoma*, *L. capverdensis*, *L. gaini*, *Lophogorgia viminalis*, *L. hebes*, *L. punicea*, and *L. violacea*. The former four all belong to the eastern Atlantic clade, while the latter three are Western Atlantic species. Further morphological and genetic analyses of these species in particular will be necessary in order to more accurately classify them and determine whether resurrecting the genus *Lophogorgia* would be appropriate.

If the South African clade is recognized as its own genus, whether through the resurrection of *Lophogorgia* or by a new name, it would complicate the taxonomy of the remaining *Leptogorgia* species. The issue is that the type species for the genus *Leptogorgia* is *L. viminalis*, formerly known as *Gorgonia viminalis* (Breedy & Guzman, 2007) is within a monophyletic group with eastern Atlantic-Mediterranean species, sister to the South African group. Therefore, if the South
African group is granted species status, then any species that are not in the sister clade with L. viminalis (which are the majority of *Leptogorgia* species) could not be classified as *Leptogorgia* and would have to be renamed. This supports Poliseno et al.’s (2017) call to reclassify almost all *Leptogorgia* species and revise the genus in its entirety, which leaves the case of *L. hebes* all the more ambiguous, as it does not fit in either *Leptogorgia* or *Lophogorgia*. It is likely that new genera need to be defined within this group to resolve these taxonomic issues.

### 4.2 Mitogenomic phylogeny

The complete mitochondrial genomes of 21 gorgonian specimens were examined to elucidate phylogenetic relationships and to test the efficacy of using complete mt genome over the single mtMutS gene. This is the first study to sequence complete mitochondrial genomes for *L. virgulata* and *L. hebes*, and the resulting mitogenomic phylogeny is in agreement with our mtMutS phylogeny and with that of Poliseno et al.’s (2017), albeit with stronger branch support. The tree topology also matches that of the mitogenomic phylogeny presented by Poliseno et al. (2017) while adding *L. hebes* and *L. virgulata* from the Gulf of Mexico as a sister clade to *E. mutabilis* and *L. alba* from the Eastern Pacific. These observations support Poliseno et al.’s conclusions that Western Atlantic gorgonians are more closely related to Eastern Pacific gorgonians than to eastern Atlantic gorgonians (*L. cf. palma*, *L. gaini*, *L. sarmentosa* and *L. capverdensis*).

### 4.3 Divergence time estimation

This is the first study to place divergence time estimates on complete mitochondrial genomes of *Leptogorgia* species. Poliseno et al. (2017) suggested the first divergence event between Eastern Pacific and Western Atlantic species occurred about 28 Ma (with error bars ranging from 12 to 45 Ma). However, the fossil-calibrated mitogenomic phylogeny presented in this study suggests that this first split between Eastern Pacific and western Atlantic species occurred later between 11 and 20 Ma (Figure 4), which is within Poliseno et al.’s (2017) lower error range. According to O’Dea et al. (2016), the formation of the Isthmus of Panama was not a singular event, but rather a series of geological events that took place over the course of the last 30 million years. Between 20 and 10 Ma, the Panama Arc island chain began to rise, based on O’Dea et al.’s (2016) estimated rates of Arc uplift. Gene flow by the exchange of gametes and larvae through the CAS was likely high up to 10 Ma, while there was still significant seawater exchange between the Atlantic and Pacific oceans (O’Dea et al., 2016). Both, *L. hebes* and *L. virgulata* are adapted to shallow water habitat ranging from 3 to 82 m (Cairns & Bayer, 2009; Williamson et al., 2011). They mature rapidly (<2 years) and are broadcasts spawners, releasing eggs and sperm into the water column (Beasley & Dardeau, 2003; Gotelli, 1991). While larval duration in *L. hebes* is not known, it can last up to 20 days in *L. virgulata* (Gotelli, 1991). These characteristics indicate a potential for high dispersal and suggest that genetic and larval connectivity likely occurred between the Pacific and Atlantic oceans through a shallow CAS. Divergence of *Leptogorgia* between these basins likely increased after 10 Ma as seawater exchange became more constricted. Our data suggests that the *L. hebes* speculated at about 11 Ma and it forms the first Western Atlantic clade on the mitogenomic tree. This divergence time coincides with the timing of more restricted water flow between the two basins.

O’Dea et al.’s (2016) uplift data show that after this uplifting period between 20 and 10 Ma, a deepening event occurred between 10 and 6 Ma, in which the Panama Arc began to drop, resulting in greater connectivity between the ocean basins. This span of time is also characterized by shifts in migration rates of both terrestrial and marine fauna, referred to as migration pulses, by Bacon et al. (2015). They specifically highlight a migration shift among marine organisms at around 7.96 Ma, based on their free model migration estimate. The synchrony of submergence of the Panama Arc and a migration event in marine organisms suggest that gene flow could have increased between the Eastern Pacific and Western Atlantic during this time. Following this period of subsidence, at around 6 Ma the Panama Arc began to emerge again and has continued to rise until the present day (O’Dea et al., 2016). Divergences of marine organisms begin to increase at this time, peaking at about 4 million years ago (O’Dea et al., 2016). This timing of events supports the divergence estimate of the Western Atlantic *Leptogorgia* sp. (KY559412) at 6.75 Ma and the divergence of the Western Atlantic *L. virgulata* clade at 5.82 Ma, both diverging from sister clades in the Eastern Pacific. The estimated times of *Leptogorgia* species divergence obtained from this study are concordant with geologic data and historic migration data (Bacon et al., 2015; O’Dea et al., 2016), supporting an initial divergence between Eastern Pacific and Western Atlantic species at about 20–11 Ma with extant lineages arising in each basin in an alternating pattern at 11 (Western Atlantic), 10 (Eastern Pacific), 7 (Western Atlantic), and 6 (Eastern Pacific) Ma (Figure 4).

The divergence times obtained from this study are more recent than those presented by Poliseno et al. (2017) and with lower error estimates (2–4 million-year range as opposed to a 12–40 million-year range). This discrepancy is most likely attributed to our use of complete mitochondrial genomes that include fourteen protein-coding genes and two RNAs instead of a single, partial gene (mtMutS). There are numerous studies of multiple taxa showing a pattern of incongruent tree topology between single mitochondrial markers and complete mitochondrial genomes despite the fact that they are the same locus and therefore share the same phylogenetic history (Havird & Santos, 2014; Knaus et al., 2011; Luo et al., 2011; Nadimi et al., 2016; Pacheco et al., 2011; Rohland et al., 2007; Urantowka et al., 2017; Wang et al., 2017; Williamslev et al., 2009). For example, Havird, Santos Scott, and Schierwater, (2014) analyze the performance of single and concatenated sets of mitochondrial genes relative to complete mitochondrial genomes for phylogenetic reconstruction of metazoans. Their findings show
that single genes are not able to reproduce the topology of a mitochondrial phylogeny (Havird & Santos, 2014). A similar study, but focusing on birds, showed that single mitochondrial genes resulted in incorrect and contradictory phylogenetic relationships, while the use of complete mitochondrial genomes accurately reflected the species tree (Urantowka et al., 2017). The same pattern has been observed in insects, where individual mitochondrial genes can result in different and contradicting tree topologies, while using the complete mitochondrial genome performs well at various taxonomic levels (Wang et al., 2017). In fungi, the phylogenetic signal differs between single mitochondrial genes, subsets of concatenated mitochondrial genes, and complete mitochondrial genomes, despite all being the same locus (Nadimi et al., 2016).

In addition to potentially generating different and contradicting tree topologies, there are numerous examples across widespread taxa on how single mitochondrial genes oftentimes result in poorly supported phylogenetic trees that become fully resolved and well supported when using complete mitochondrial genomes (i.e., Arquez, Colgan, & Castro, 2014; Justice, Weese, & Santos, 2016; Perseke, Golombek, Schлегel, & Struck, 2013; Williams, Foster, & Littlewood, 2014; Yu, Li, Ryder, & Zhang, 2007). Phylogenies in Octocorals present a similar issue; previous research shows that it is difficult to distinguish between species when using the single gene mtMutS and that even using a concatenated set of 2–3 different mitochondrial regions only allows to distinguish 70%–80% of morphological species (i.e., Baco & Cairns, 2012; McFadden et al., 2011). The low resolution provided by the use of a single mitochondrial region explains the low support for many clades in the mtMutS phylogeny presented in this study and that of Poliseno et al. (2017). Greater resolution and strong support of clades within the Octocorallia is achieved by using complete mitochondrial genomes, as demonstrated in our present study and in previous research (i.e., Figueroa & Baco, 2015, 2014; Kayal et al., 2013; Poliseno et al., 2017).

In addition to incongruent topologies and weakly supported clades, the use of single genes can result in overestimation of calibrated divergence times (Duchêne et al., 2011; McCormack et al., 2011). McCormack et al. (2011) demonstrates that divergence estimation from single mitochondrial genes results in earlier divergence times compared to the use of several markers from the mitochondrial and nuclear genome. They show that the gene tree reconstructed from single mitochondrial markers is not as robust and differs from the species tree reconstructed by using multiple markers from various loci (McCormack et al., 2011). While McCormack et al. (2011) did not examine if complete mitochondrial genomes alone would yield better results, similar to those obtained when using several mitochondrial and nuclear markers, the research by Duchêne et al. (2011) suggests that this might be the case. In their study, Duchêne et al. (2011) compared phylogenetic divergence estimates for cetaceans based on single mitochondrial genes, different combinations of concatenated genes, and complete mitochondrial genomes. Their results show that tree topology from single genes can differ from each other due to different substitution rates and that single gene divergence time estimates consistently resulted in overestimation of divergence times when compared to the use of complete mitochondrial genomes (Duchêne et al., 2011). These results from previous research are congruent with our observations that in octocorals, such as the gorgonians analyzed in our study, the use of complete mitochondrial genomes as opposed to single mitochondrial genes, results in better resolved, well supported, trees that have earlier and more precise divergence time estimates. Since our divergence time estimates are concordant with regional geological events and divergence patterns of other organisms, it supports our hypothesis that the divergence times of Eastern Pacific and Western Atlantic Leptogorgia lineages is younger than previously suggested (Poliseno et al., 2017) with the majority of speciation events occurring after 10 Ma when significant seawater exchange between the Pacific and Atlantic Ocean ceased (e.g., Bacon et al., 2015; Montes et al., 2015; O’Dea et al., 2016). However, future work that includes multiple nuclear markers in addition to mitochondrial genomes is necessary to fully test this hypothesis.

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CONFLICT OF INTEREST
The authors declare they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

DFF conceived the ideas and designed methodology; DH collected the specimens; SS, NJF, and DFF generated the genetic data; SS and NJF analyzed the data; DFF and DH supervised research and analyses; SS and DFF wrote the manuscript; SS, DFF, NJF, and DH contributed to the interpretation of data. All authors contributed critically to the drafts and gave final approval for publication.

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OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://www.ncbi.nlm.nih.gov/genbank/.

DATA AVAILABILITY STATEMENT

Mitochondrial genome and mtMutS sequences can be accessed online through GenBank (accession numbers listed in Table 4).

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