Range extension of *Mesophyllum erubescens* (Foslie) Me. Lemoine (Hapalidiales, Rhodophyta): first report from mesophotic rhodolith beds in the northwestern Gulf of Mexico offshore Louisiana and Texas, including the Flower Garden Banks National Marine Sanctuary

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**Abstract**
DNA sequence analysis of plastid-encoded *psbA* and UPA, mitochondrion-encoded COI, and nuclear-encoded LSU rDNA of rhodolith-forming crustose coralline algal specimens from the northwestern Gulf of Mexico reveals that *Mesophyllum erubescens* (Foslie) Me. Lemoine is present in mesophotic rhodolith beds offshore Louisiana and Texas at 39–57 m depth. Morpho-anatomical characters viewed with SEM support the identification of these specimens. *Mesophyllum erubescens* is reported for the first time offshore Louisiana at Ewing Bank, the Louisiana–Texas border at Bright Bank, and Texas in the Flower Garden Banks National Marine Sanctuary.

**Keywords**
CCA, COI, coralline algae, DNA, LSU rDNA, *psbA*, UPA.

**Introduction**
Previously, species identification and reports of coralline algal diversity in the northwestern Gulf of Mexico (NWGMx) have been based largely on morpho-anatomy. Few floristic studies of coralline algae were conducted in the region prior to 2014 (Minnery et al. 1985; Rezak 1985; Minnery 1990; Fredericq et al. 2009). Recently, DNA sequence-based floristic surveys and biodiversity assessments of crustose coralline algae (CCA), including rhodoliths, have begun in the region (Richards et al. 2014, 2016). Though two new coralline species have been described from the region (Richards and Fredericq 2018; Richards et al. 2019), many other new species have yet to be described. Also, other species described from localities outside the region likely remain to be identified.
from mesophotic collections in the NWGMx. Herein, we focus on documenting the presence of a described species that has yet to be reported from the region, *Mesophyllum erubescens* (Foslies) Me. Lemoine, whose identification has recently been clarified by comparative DNA sequence analyses including the holotype specimen (Sissini et al. 2014).

**Methods**

Rhodolith-forming specimens were collected (Table 1) offshore Louisiana and near the Texas–Louisiana border aboard the R/V *Pelican* using an Hourglass-design box dredge with minimum tows (Joyce and Williams 1969) as described in Richards et al. (2019) and from offshore Texas in the Flower Garden Banks National Marine Sanctuary (FGBNMS) via the ROV *Mohawk* aboard the R/V *Manta*, as described in Fredericq et al. (2019). Brazilian and Hawaiian specimens were collected from Arvoredo Island, Santa Catarina, southern Brazil, and at Richardson Ocean Park, Hilo, Hawai‘i, respectively, to provide comparative loci among Brazilian, Hawaiian, and NWGMx specimens. A distribution map was generated in R using package “ggplot2” (Wickham 2016; R Core Team 2019).

DNA was extracted, amplified, and sequenced as described in Richards et al. (2014, 2016). The markers chosen for PCR included the plastid-encoded *psbA* (photosystem II reaction center protein D1 gene) and UPA (partial 23S rDNA), mitochondrial-encoded COI (cytochrome oxidase subunit I gene), and nuclear-encoded LSU (partial 28S rDNA). *Mesophyllum erubescens* sequences were initially identified using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990, 2000) as described in Richards et al. (2017). Published sequences were also downloaded from GenBank and included in the analysis for context, including sequences of *M. erubescens* that were verified by comparisons to *Phymatolithon calcareum* as the outgroup. The final four-gene concatenated alignment was 2,414 base pairs (bp) long (*psbA* = 831 bp; UPA = 370 bp; COI = 613 bp; LSU = 600 bp). The alignment was analyzed for maximum likelihood (ML) using RAxML (Stamatakis 2006) implemented via “The CIPRES Science Gateway v. 3.3” (Miller et al. 2010) as described in Richards et al. (2017). Sequence divergence analyses were conducted in MEGA 5.2.2 (Tamura et al. 2011) by calculating p-distance. Alignments were cropped at the 5’ and 3’ ends prior to divergence analyses to remove missing data. Scanning electron microscopy (SEM) was performed as described in Richards et al. (2016).

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<th>Latitude</th>
<th>Longitude</th>
<th>Date</th>
<th>Depth (m)</th>
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**Table 1.** List of collection information, GenBank numbers, and reference information for sequences of taxa included in phylogenetic analyses.
Results

*Mesophyllum erubescens* (Foslie) Me. Lemoine

**New records.** USA; offshore Louisiana; Ewing Bank; 23°05.654’N, 091°01.646’W; 56 m; 16 May 2019; collected by S. Fredericq, W.E. Schmidt, R.P. Kittle III; 2 individuals (vouchers LAF 7380, LAF 7385, tetrasporophytes). USA; offshore Louisiana–Texas border; Bright Bank; 27°53.660’N, 093°18.620’W; 56–57 m; 16 May 2019; collected by S. Fredericq, W.E. Schmidt, R.P. Kittle III; 1 individual (voucher LAF 7386). USA; offshore Louisiana–Texas border; Bright Bank; 27°53.418’N, 093°18.219’W; 39 m; 25 May 2000; collected by S. Fredericq, Brigitte Gavio; 1 individual (voucher LAD1939). USA; offshore Texas; East Flower Garden Bank; 27°56.314’N, 093°36.027’W; 52 m; 24 September 2018; collected by S. Fredericq, J.L. Richards, R.P. Kittle III, Dijel Fuselier; 1 individual (voucher LAF 7326, tetrasporophyte). USA; offshore Texas; East Flower Garden Bank; 27°56.314’N, 093°36.027’W; 53 m; 18 September 2019; collected by S. Fredericq, J.L. Richards; 1 individual (voucher LAF 7459, tetrasporophyte). USA; Hawai‘i; Hawai‘i Island; Richardson Ocean Park, Hilo; 19°44.15’N, 155°00.833’W; 1–2 m (collected by snorkeling); 26 May 2013; collected R.P. Kittle III; 1 individual (voucher LAF 6921, tetrasporophyte). See Table 1 for collection information and GenBank accession numbers corresponding to specimen voucher numbers and Figure 1 for distribution map.

**Identification.** The identification of specimens was confirmed by analysis of DNA sequences (Fig. 2), including sequences of specimens whose identifications were previously verified by comparisons to type material. *PsbA* sequences of NWGMx specimens were 0–0.1% divergent from sequences of Brazilian specimens, and NWGMx and Brazilian specimens were 0.3–0.4% divergent from the Hawaiian specimen. UPA sequences of the NWGMx specimens were identical to the Brazilian specimens, and 0.3% divergent from the Hawaiian specimens. LSU sequences of the NWGMx, Brazilian, and Hawaiian specimens were all identical to each other. COI sequences of the NWGMx specimens were 3.4–3.7% divergent from the Hawaiian specimens.

Observation of habit and SEM imaging (Fig. 3) showed characters congruent with *M. erubescens*, e.g., protuberant habit (Fig. 3A), presence of cell fusions (Fig. 3B), lack of secondary pit connections, a single layer of non-armored (i.e., not “flared”) epithallial cells (Fig. 3C), and abundant multporate conceptacles (Fig. 3D–G). Conceptacle roofs flat to slightly rounded (Fig. 3D, F, G.), with pore canals showing apical pore plugs (Fig. 3E). Longitudinal sections of protuberances showed radial construction and layers of multporate conceptacles that became infilled with adventitious vegetative cells after spore release (Fig. 3F, G).

**Discussion**

Results of the ML analyses (Fig. 2) show *Mesophyllum erubescens* is present in mesophotic rhodolith beds offshore Louisiana and Texas in the NWGMx. Specimens were collected at Ewing Bank, Bright Bank, and East Flower Garden Bank in the FGBNMS. These are the first confirmed reports of *M. erubescens* in the northern Gulf of Mexico. Minnery (1990) reported *Mesophyllum* sp. from the FGBNMS, but did not list a specific epithet. The image in Minnery (1990: 998, fig. 5I), shows an algal nodule from West Flower Garden Bank that may correspond to *M. erubescens*, considering that the image shows layers of overgrown conceptacles infilled with adventitious vegetative cells. *Mesophyllum erubescens*
was recently placed in a new genus, *Melyvonnea* Athanasiadis & D.L. Ballantine, along with a few other species of *Mesophyllum* (Athanasiadis and Ballantine 2014). Because there are no DNA sequences available of the generitype, *Melyvonnea canariensis*, we here retained *M. erubescens* in *Mesophyllum* pending further molecular evidence.

Reports based on DNA sequence analyses show that the known distribution of *Mesophyllum erubescens* currently includes Brazil, the southwestern Gulf of Mexico (SWGMx), the NWGMx, and Hawai‘i (Fig. 1) (Sherwood et al. 2010; Sissini et al. 2014; Jesionek et al. 2016; present study). Sissini et al. (2014) noted that the identification of the Hawaiian population of *M. erubescens* needs confirmation with *psbA*, *rbcL*, or COI. We show here in this study that *psbA* sequences of the Brazilian and NWGMx specimens were only 0.3–0.4% divergent (2–3 base pairs) from the Hawaiian specimen, suggesting that the Hawaiian population is conspecific with *M. erubescens*. For context, previous phylogenetic studies of taxa within the Hapalidiales have reported minimum interspecific divergence values of 0.7% or greater for *psbA* (Adey et al. 2015; Jesionek et al. 2020). However, considerable divergence was observed for the COI marker indicating that further studies need to be performed to clarify the relationship between the Pacific and Atlantic populations of this species. COI sequences were 3.4–3.7% divergent between NWGMx and Hawaiian specimens, which is greater than the maximum intraspecific divergence value of 1.8% reported in a previous study for *Phymatolithon* spp. (as “infralineage variation”; Peña et al. 2015). However, Peña et al. (2014) reported that intraspecific divergence (as “infralineage variation”) between *Mesophyllum* spp. was as high as 3.24%.

Observation of habit and SEM imaging (Fig. 3) supports the identification of these NWGMx specimens and showed characters congruent with the *Mesophyllum erubescens* description (Sissini et al. 2014). It is important to note that although these characters support the species identification, the morpho-anatomical characters alone can not be used to identify this species, because the characters are shared by other members of the Hapalidiales. For example, both *M. erubescens* and *M. sphaerium* tetrasporophytes possess protuberances with radial construction and abundant multiporate conceptacles with flat roofs that become overgrown after spore release.

**Figure 2.** Maximum likelihood phylogenetic analyses of concatenated *psbA*, UPA, COI, and LSU (2,414 bp) DNA sequences of *Mesophyllum erubescens* and *Mesophyllum sphaerium*, with *Phymatolithon calcaratum* as outgroup. Sequences shown in bold are newly generated in this study. Numbers at nodes are bootstrap values (1,000 replicates). NWGMx = northwestern Gulf of Mexico; SWGMx = southwestern Gulf of Mexico.
Figure 3. *Mesophyllum erubescens*. A. Thallus habit of northwestern Gulf of Mexico specimens. Top (left to right): LAF 7385, LAF 7326, LAF D1939. Bottom (left to right): LAF 7380, LAF 7459, LAF 7386. B. Perithallum with cell fusions (arrows). C. Partial surface view and section view showing perithallium (bracket), intercalary meristematic cells (*), and epithallial cells (white arrows), one with intact epithallial cell roof (black arrow). D. LAF 7326. Surface view of protuberance with multiporate conceptacles (arrows). E. LAF 7326. Conceptacle pores with intact apical pore plugs (circle pointers). F. LAF 7326. Longitudinal fracture of protuberance showing conceptacles (arrowheads), one infilled with adventitious vegetative cells (right arrowhead). G. LAF 7380. Longitudinal fracture of protuberance with layers of overgrown conceptacles infilled with adventitious vegetative cells. Scale bars: A = 2.5 cm; B = 27.5 µm; C = 11 µm; D = 280 µm; E = 14 µm; F = 150 µm; G = 400 µm.
Interestingly, empty conceptacles of *M. erubescens* become infilled with adventitious vegetative cells after spore release (Fig. 3D, E), which is different from other members of the Hapalidiales present in the NWGMx that become infilled with aragonite crystals (e.g. *Lithothamnion* spp.; Kraysky-Self et al. 2016). The findings in this study help to better delineate the true diversity and define the precise taxonomic composition of crustose coralline red algal flora of the Flower Garden Banks National Marine Sanctuary and offshore Louisiana.

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**Authors’ Contributions**

JLR, RPK, and SF conceived the idea for the project. JLR, RPK, DF, WES, CFDG and SF assisted in specimen collection. JLR, JA, CFDG generated SEM data; JLR, RPK, JA, and DF generated DNA sequence data. JLR, RPK, JA, DF and WES analyzed and interpreted the data. RPK generated the distribution map. JLR, RPK, CFDG, WES, and SF wrote the manuscript. All authors critically reviewed the final manuscript.

**References**


Richards et al. | Mesophyllum erubescens in the NWGMx


