A Dynamic Approach to the Study of Rhodoliths: a Case Study for the Northwestern Gulf of Mexico

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Abstract – In the northwestern Gulf of Mexico, beds of rhodoliths and unconsolidated rubble at 55-70 m depth are associated with unique offshore deep bank habitats known as salt domes or diapirs. Prior to the 2010 BP Deepwater Horizon oil spill these harbored the highest known seaweed diversity in the northern Gulf of Mexico. Six post-spill cruises led offshore Louisiana to two sites previously documented with rich algal assemblages (*i.e.* pre-spill) revealed a dramatic post-spill die-off of seaweeds at both sites, with dredged rhodoliths appearing bleached and mostly denuded of fleshy algae, or "bare" (with a few crustose genera only, e.g. Corallinales and Peyssonneliales). This rubble, brought to the laboratory and maintained in a series of ~75 liter microcosm tanks, gradually became covered by a suite of red, green and brown seaweed germlings that to this day continue to grow to adult size, reproduce, disappear and re-emerge, and whose species composition reflects pre-spill assemblages. These experiments revealed the expression of biodiversity from alternative life stages or resting stages apparently repressed in the Gulf at the time of sampling, including new, previously overlooked diversity. The rate of algal succession was documented by biweekly photography, and species taxonomic identity is being confirmed by ongoing molecular and morphological evidence. The implications of these exciting results, namely that undetected propagules, spores and endolithic filaments collected along with the "bare" substrata and *in situ* seawater have been triggered to germinate, grow, and reproduce under laboratory conditions are far-reaching. We hypothesize the function of rhodoliths and rubble as marine seedbanks for biological diversity and explore the role of this ecosystem for community resilience following a major anthropogenic disaster. This is a speculative paper since we currently lack many rigorous, quantitative data. The paper is envisioned as a "first step" in approaching the dynamics of rhodoliths and associated diversity following a catastrophic anthropogenic event, from which the algal and invertebrate diversity has not recovered, as of October 2013, our last collecting expedition to Ewing Bank in the NW Gulf of Mexico.

Benthic / biodiversity / Deepwater Horizon / Gulf of Mexico / macroalgae / oil spill / rhodoliths / salt domes / seaweeds

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INTRODUCTION

Rhodoliths are free-living marine nodules of various sizes that are composed of crustose (non-geniculate) coralline red algae precipitating calcium carbonate (Foster, 2001) or are covered by a suite of other encrusting algae. We here refer to "unconsolidated rubble" as rhodolith nodules that are bare or partially algal-denuded. Rhodoliths in the northwestern Gulf of Mexico (NWGMx, Fig. 1) are associated with unique deep bank habitats on the outer continental shelf, the salt domes or diapirs that are peculiar to that part of the northern Gulf at depths of 55-70 m (Rezak *et al.*, 1985; Fredericq *et al.*, 2009). More than 500 of these salt domes are presently recorded, spread in a series across a large area extending from SE of Dallas, Texas, across NW Louisiana, SE Mississippi and just N of Mobile, Alabama, USA (Gore, 1992). A major category of rhodoliths associated with the salt domes is autogenic in origin *i.e.* established by differential erosion processes of diapir salt (halite) with the ensuing rubble surface becoming secondarily settled by both encrusting and fleshy algae. Whereas salt domes are almost 100% pure salt or halite, very small amounts of impurities, notably $CaSO_4$ (anhydrite and gypsum) also occur. If underground water dissolves the salt away, the remaining anhydrite forms a somewhat insoluble barrier that is then acted upon by anaerobic bacteria that convert $CaSO_4$ to $CaCO_3$ resulting in autogenic calcium carbonate (rhodolith) nodules. Anaerobic bacteria obtain the carbon necessary to reduce anhydrite to limestone from hydrocarbons in petroleum products that accumulate in crevices and pockets along the edge of the offshore salt dome banks on the continental shelf (Gore, 1992; Rezak *et al.*, 1985). The composite sedimentary overlayer of $CaCO_3$, gypsum, and anhydrite above a diapir is known geologically as caprock and ranges in thickness from 1 to over 450 m. It requires approximately 600 m of salt to be dissolved to produce 1/3m of caprock (Gore, 1992). The other category of rhodoliths in the NW Gulf of Mexico is biogenic in origin and formed by the organisms themselves, *i.e.* non-geniculate coralline red algae. It is the banks' caprock slopes and peaks that today are capped by both autogenic and biogenic rhodoliths.

By virtue of their geological history, especially in that they often surmount salt domes where strata trap hydrocarbons, many of these rubble habitats are located in or immediately adjacent to areas of intensive oil and gas exploration (Fig. 1), production and transportation. Although these deep bank rubble habitats are prominent features harboring the highest known diversity of seaweeds in the Northern Gulf (Figs 2, 3) (e.g., Gavio *et al.* 2005), knowledge about the extent of rhodolith beds in the NWGMx is gravely deficient.

Interpretation of sediment texture data in the NWGMx where clastic terrigenous sediments are found in association with biogenic carbonate sediments is not straightforward. Unconsolidated rubble/nodules that have a high percentage of $CaCO_3$ (> 60%) are assumed to be mainly biogenic in origin. Four aspects of algal nodules that have been considered as environmentally significant parameters and indicators of depositional environments are shape, coralline-algal growth form, biotic composition, and diagenetic alteration (Minnery, 1990). Shape, growth form, and biotic composition were used as evidence that nodules at the edge of thriving coral reefs, *i.e.* the Flower Garden Banks National Marine Sanctuary (FGBNMS) on the outer continental shelf about 170 km due south of the Texas-Louisiana border, represent two generations of growth: one in shallow water during the early stages of the post-glacial transgression and a second,

modern, deep-water stage. The tops of these northernmost tropical coral reefs in the US (with the reefs themselves topping salt domes) rise above the surrounding seafloor, some to within 20m of the water surface, and extensive rhodolith beds are found below the hermatypic coral zone.

Identification of Corallinales-dominated rhodoliths at the species level in the NW Gulf of Mexico is not yet possible because available names need to be validated by comparisons to type specimens. So far nine species are recognized, including *Lithothamnion* spp. and *Mesophyllum* spp. *sensu lato* (Melobesiodeae, Hapalidaceae), *Lithophyllum* sp. (Lithophylloideae, Corallinaceae) and
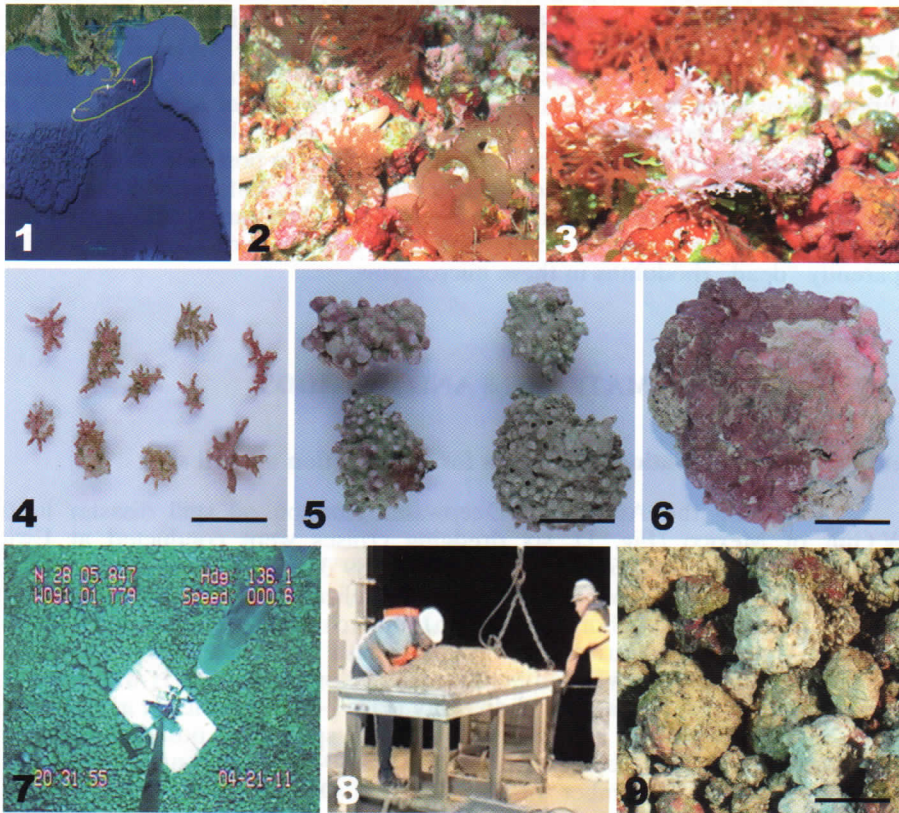
Figs 1-9. **1.** Map of the northwestern Gulf of Mexico showing the demarcated area representing the five post-Deepwater Horizon spill collection sites (*i.e.* Ewing Bank, Sackett Bank). **2-3.** NW Gulf of Mexico showing *in situ* rhodoliths (Corallinales, Peyssonneliales) bearing a lush assemblage of epiphytic fleshy algae prior to the DWH oil spill, ~70 m depth. Photo courtesy of Emma Hickerson, FGBNMS. **4.** *Lithothamnion* rhodoliths, collected from Ewing Bank, pre-DWH oil spill, ~65 m depth. Scale = 2 cm. **5.** Biogenic coralline rhodoliths, collected from Ewing Bank, pre-DWH oil spill, covered with green algal borers, ~65 m depth. Scale = 1.75 cm. **6.** Autogenic rubble covered with a mixture of Corallinales and Peysonneliaceae, Ewing Bank, pre-DWH oil spill, ~65m depth. Scale = 3 cm. **7.** Post-spill Rhodolith beds, Ewing Bank, photographed *in situ* with Seaviewer Camera, 4-21-2011, 28°05.847'W, 91°01.779'W, 60m depth. **8.** Bare rubble dredged on 12/4/2010 and loaded aboard the *R/V Pelican*; oil fumes were strong at one site at Ewing Bank, 55 m depth. **9.** Bare rubble and partly algal-denuded rhodoliths, dredged 12/4/2010 at Ewing Bank, ~66m depth.

Porolithon spp. (Porolithoideae, Corallinaceae) (J. Richards, in prep.). Although the shape of a single rhodolith is widely standardized (e.g., ball-shaped, laminated, nodular) (Bosence & Pedley, 1982), a single rhodolith "stone" can comprise several overlapping crustose species that are phylogenetically unrelated (Basso, 1998; Yabur-Pacheco *et al.*, 1982). Many rhodoliths in the NW Gulf are such hybrids, with a single rhodolith composed for example of a mix of crustose corallines and Peyssonneliaceae (Fig. 6), and the encrusting stage of the brown alga *Lobophora* spp. (Dictyotaceae) (pers. obs.).

This account builds heavily on pre-Deepwater Horizon (DWH) oil spill inventory baseline data characterizing the assemblages of seaweeds growing on rhodoliths and unconsolidated rubble living primarily on hard banks 55-70 m deep throughout the NWGMx in areas under assumed threat of increasing offshore oil and gas development. Algal collections before the April 2010 DWH oil spill disaster were critical to our contributions (Fredericq *et al.*, 2009) in a recently published comprehensive account of Gulf of Mexico biodiversity (Felder & Camp, 2009), the baseline for understanding diverse algal and faunal assemblages now at risk. We here report qualitatively on our observations pertaining to post-spill dredged rubble brought back from Ewing Bank to our laboratory at UL Lafayette as "live rocks" where to this day they are maintained in a series of 75 liter tanks, and introduce the novel concept of seedbanks to account for the resurgence of algal diversity from apparently "dead" rhodoliths in these microcosms.

MATERIALS AND METHODS

Fieldwork and preparation of samples for preservation aboard ship

Following the April 2010 Deepwater Horizon oil spill disaster in the NW Gulf of Mexico (Fig. 1), six dredging cruises were conducted at Ewing Bank (28°05.737'N; 91°01.608'W; 28°38.019'N; 89°33.262'W) and Sackett Bank (~28°38.019'N; 89°33.262'W) aboard the *R/V* Pelican, the UNOLS (University-National Oceanographic Laboratory System) research vessel stationed at LUMCON (Louisiana Universities Marine Consortium). These two banks offshore Louisiana were chosen because they were oil-impacted sites close to the DWH well explosion and exceptionally rich in macroalgal diversity (Figs 2-3) (Fredericq *et al.*, 2009) and rhodolith types (Figs 4-6) before the DWH oil spill. The post-spill collecting expedition dates were December 2-6, 2010; April 19-24, 2011; August 26-30, 2011; August 24-26, 2012; November 15-17, 2012; and October 16-20, 2013. Post-spill dredging in rhodolith/rubble substrata (Fig. 7) involved a vessel-deployed Hourglass-design box (Fig. 8) using two periods less than 10 minutes (Joyce *et al.*, 1969).

Dredged rhodoliths/rubble (Figs 8-12) at each station were placed in separate ziploc bags and vials, preserved in 5% diluted formalin/seawater for morphological studies, and in the desiccant silica gel for molecular phylogenetic analyses.

Preparation of samples for microcosm studies

Bare or partly algal-denuded unconsolidated rubble, including many small rhodoliths, were dredged in two oil-impacted sites, Ewing Bank and Sacket Bank in April and August 2011, and August and November 2012, and stored by location in

coolers and containers filled with on-site seawater kept aerated. Water was collected *in situ* at same sampling depths using a CTD/Rosette system with Niskin bottles, along with physico-chemical readings. In order to ensure a more complete characterization of the community following NWGMx field collection and return to UL Lafayette, samples from each site were divided with a randomized arrangement, labeled, photographed and used to establish microcosms in closed 20-gallon closed system tanks each fitted with a protein skimmer and 600 lumen lights, maintained in approximately 10 hrs light /14 hrs dark cycle, and a standard FRITZ f/2 growth media nutrient regimen (Fritz Industries, inc. Dallas TX, USA); we added 1/2 of the manufacturer's recommendation, 5 ml/75 liter every three months. Only seawater retrieved from sampled depths in the field, and augmented periodically by DI water to counteract evaporation, was used in the microcosms. The temperature of the water in the tanks remains constant at about 24°C, the same temperature measured in the field at 55 m depth in late summer (pers. obs). PAR and carbonate chemistry measurements are not available. The rate of algal succession was photographed every two weeks (Figs 23-28). Visible developing algae were subsampled and preserved for morphological and molecular study.

Voucher specimen treatment at UL Lafayette

Algal collections from each trip were sorted, identified to species level, archived and deposited at the University of Louisiana at Lafayette Herbarium (LAF) using standard archiving protocols (Tsuda & Abbott, 1984).

DNA extraction, barcoding and phylogenetic analyses

The families Peyssonneliaceae (Peyssonneliales) and Rhizophyllidaceae (Dumontiaceae-complex) collected in the Gulf of Mexico were selected for in depth phylogenetic study because they comprise two assemblages of non-coralline crustose red algae covering unconsolidated rubble forming rhodoliths. DNA extractions were performed on silica gel-preserved samples, herbarium material and live samples using the DNeasy Plant Mini Kit (Qiagen Valencia, CA) following the manufacturer's instructions. Samples included 62 specimens of the Peyssonneliaceae and 20 specimens of the Rhizophyllidaceae (Dumontiaceae-complex) (Table 1).

PCR for the chloroplast-encoded *rbc*L gene was conducted using the methods and primers referenced in Cho *et al.* (2003). Resulting PCR products were sequenced both in-house on an ABI Model 3130xl Genetic Analyzer and commercially (by Beckman Coulter Genomics Danvers, MA). Chromatograms were assembled using Sequencher 5.1 (Gene Codes Corp., Ann Arbor, MI, USA) and aligned manually with MacClade 4.08 (Maddison & Maddison, 2000). Using two Rhizophyllidaceae outgroup taxa for the Peyssonneliaceae dataset, and two Gigartinaceae for the Dumontiaceae-complex dataset, the two dataset alignments were each analyzed in PartitionFinder (Lanfear *et al.*, 2012) to determine the best partition scheme and model(s) of evolution as can be implemented by RAxML. For both data sets, a three codon position partitioning scheme, each evolving with GTR +I+ G model was selected on the basis of the Akaike information criterion (AIC), corrected Akaike information criterion (AICc), and Bayesian information criterion (BIC). The alignment with the above models and partitioning scheme was then analyzed for Maximum Likelihood (ML) with RAxML v 2.4.4 (Stamatakis, 2006) and 1000 ML bootstrap replicates in order to assess branch support.

Table 1. List of taxa studied, identification number (Id. no.), locality information, collector, GenBank accession number (earlier sequences derived from Fredericq lab, with * = newly submitted)

Taxa	Id. No.	Locality	Collector	GenBank
Riquetophycus clade	K84	Dry Tortugas, FL, USA	S. Fredericq, 31 May 2004	EU349158
"*Metapeyssonnelia corallepida*"	K85	Dry Tortugas, FL, USA	S. Fredericq, 31 May 2004	EU349214
"*P.*" *inamoena* clade	K86	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349116
"*P.*" *boergesenii* clade	K87	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349146
"*P.*" *inamoena* clade	K100	Dry Tortugas, FL, USA	S. Fredericq, 31 May 2004	EU349134
"*Polystrata fosliei*"	K101	Dry Tortugas, FL, USA	S. Fredericq, 31 May 2004	EU349211
Riquetophycus clade	K102	Dry Tortugas, FL, USA	S. Fredericq, 01 Jun 2004	EU349160
New Genus Clade	K103	Dry Tortugas, FL, USA	S. Fredericq, 01 Jun 2004	EU349151
"*P.*" *inamoena* clade	K104	Dry Tortugas, FL, USA	S. Fredericq, 01 Jun 2004	EU349132
"*P.*" *inamoena* clade	K105	Dry Tortugas, FL, USA	S. Fredericq, 01 Jun 2004	EU349117
"*P.*" *inamoena* clade	K106	Dry Tortugas, FL, USA	S. Fredericq, 01 Jun 2004	EU349128
Riquetophycus clade	K107	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349161
"*Rhizophyllis pacifica*"	K108	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349198
"*P.*" *nordstedtii*	K109	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349149
New Genus Clade	K110	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349153
Ramicrusta clade	K111	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349193
"*Polystrata fosliei*"	K112	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349212
"*P.*" *inamoena* clade	K113	Dry Tortugas, FL, USA	S. Fredericq, 02 Jun 2004	EU349118
"*P.*" *inamoena* clade	K114	Dry Tortugas, FL, USA	S. Fredericq, 03 Jun 2004	EU349119
"*P.*" *inamoena* clade	K115	Dry Tortugas, FL, USA	S. Fredericq, 03 Jun 2004	EU349120
Riquetophycus clade	K116	Dry Tortugas, FL, USA	S. Fredericq, 03 Jun 2004	EU349162
Ramicrusta clade	K117	Dry Tortugas, FL, USA	S. Fredericq, 03 Jun 2004	EU349196
"*P.*" *inamoena* clade	K120	Louisiana-Texas Plateau, USA	S. Fredericq, 27 May 2000	EU349125
"*P.*" *inamoena* clade	K121	Louisiana-Texas Plateau, USA	S. Fredericq, 26 May 2000	EU349130
"*P.*" *inamoena* clade	K122	Louisiana-Texas Plateau, USA	S. Fredericq, 01 Jul 2001	EU349135
Ramicrusta clade	K134	Campeche Banks, MEXICO	S. Fredericq, 10 Jun 2005	EU349194
"*P.*" *boergesenii* clade	K135	Campeche Banks, MEXICO	S. Fredericq, 09 Jun 2005	EU349145
"*Contarinia magdae*"	K136	Campeche Banks, MEXICO	S. Fredericq, 17 Jun 2005	EU349201
New Genus Clade	K137	Campeche Banks, MEXICO	S. Fredericq, 17 Jun 2005	EU349152
"*Rhizophyllis pacifica*"	K138	Campeche Banks, MEXICO	S. Fredericq, 23 Jun 2005	EU349199
"*P.*" *inamoena* clade	K139	Campeche Banks, MEXICO	S. Fredericq, 23 Jun 2005	EU349123
Contarinia peyssonneliaeformis	K149	Campeche Banks, MEXICO	S. Fredericq, 11 Jun 2005	EU349200
"*P.*" *nordstedtii*	K150	Campeche Banks, MEXICO	S. Fredericq, 11 Jun 2005	EU349150
"*P.*" *inamoena* clade	K151	Campeche Banks, MEXICO	S. Fredericq, 22 Jun 2005	EU349131
"*P.*" *inamoena* clade	K152	Campeche Banks, MEXICO	S. Fredericq, 22 Jun 2005	EU349126
Riquetophycus clade	K153	Campeche Banks, MEXICO	S. Fredericq, 09 Jun 2005	EU349166
SW sp. nov. 2	K154	Campeche Banks, MEXICO	S. Fredericq, 04 Jun 2005	EU349203
"*Polystrata fosliei*"	K155	Campeche Banks, MEXICO	S. Fredericq, 14 Jun 2005	EU349213
"*P.*" *boergesenii* clade	K156	Campeche Banks, MEXICO	S. Fredericq, 14 Jun 2005	EU349147
"*Contarinia magdae*"	K157	Campeche Banks, MEXICO	S. Fredericq, 14 Jun 2005	EU349202
SW sp. nov. 2	K158	Campeche Banks, MEXICO	S. Fredericq, 10 Jun 2005	EU349204
Riquetophycus clade	K159	Campeche Banks, MEXICO	S. Fredericq, 13 Jun 2005	EU349165
"*P.*" *inamoena* clade	K160	Campeche Banks, MEXICO	S. Fredericq, 06 Jun 2005	EU349127
Riquetophycus clade	K161	Campeche Banks, MEXICO	S. Fredericq, 10 Jun 2005	EU349164
Riquetophycus clade	K162	Campeche Banks, MEXICO	S. Fredericq, 22 Jun 2005	EU349155
"*P.*" *inamoena* clade	K174	Louisiana-Texas Plateau, USA	S. Fredericq, 28 Jun 2006	EU349137
Riquetophycus clade	K175	Louisiana-Texas Plateau, USA	S. Fredericq, 28 Jun 2006	EU349172

Table 1. List of taxa studied, identification number (Id. no.), locality information, collector, GenBank accession number (earlier sequences derived from Fredericq lab, with * = newly submitted) *(continued)*

| Taxa	Id. No.	Locality	Collector	GenBank
"P." inamoena clade	K176	Louisiana-Texas Plateau, USA	S. Fredericq, 28 Jun 2006	EU349127
NE sp. nov. 1	K179	Florida Middle Grounds, USA	S. Fredericq, 03 Jul 2006	*KJ162093
"P." inamoena clade	K180	Florida Middle Grounds, USA	S. Fredericq, 03 Jul 2006	*KJ162096
"P." inamoena clade	K181	Florida Middle Grounds, USA	S. Fredericq, 03 Jul 2006	EU349121
NE sp. nov. 1	K182	Florida Middle Grounds, USA	S. Fredericq, 03 Jul 2006	*KJ162094
Riquetophycus clade	K183	Florida Middle Grounds, USA	S. Fredericq, 04 Jul 2006	EU349170
"*Rhizophyllis pacifica*"	K184	Florida Middle Grounds, USA	S. Fredericq, 04 Jul 2006	EU349197
Riquetophycus clade	K185	Florida Middle Grounds, USA	S. Fredericq, 04 Jul 2006	EU349169
Ramicrusta clade	K186	Florida Middle Grounds, USA	S. Fredericq, 04 Jul 2006	EU349195
Riquetophycus clade	K188	Florida Middle Grounds, USA	S. Fredericq, 05 Jul 2006	EU349154
Ramicrusta clade	K189	Florida Middle Grounds, USA	S. Fredericq, 05 Jul 2006	*KJ162101
"*Rhizophyllis pacifica*"	K190	Florida Middle Grounds, USA	S. Fredericq, 05 Jul 2006	*KJ162087
Ramicrusta clade	K191	Florida Middle Grounds, USA	S. Fredericq, 05 Jul 2006	EU349191
"P." inamoena clade	K194	Florida Middle Grounds, USA	S. Fredericq, 06 Jul 2006	EU349122
Riquetophycus clade	K195	Louisiana-Texas Plateau, USA	S. Fredericq, 08 Jul 2006	EU349157
"*Rhizophyllis pacifica*"	K196	Louisiana-Texas Plateau, USA	S. Fredericq, 08 Jul 2006	*KJ162089
Riquetophycus clade	K198	Louisiana-Texas Plateau, USA	S. Fredericq, 09 Jul 2006	*KJ162107
Rhizophyllis pacifica	K200	Louisiana-Texas Plateau, USA	S. Fredericq, 09 Jul 2006	*KJ162088
"P." inamoena clade	K201	Louisiana-Texas Plateau, USA	S. Fredericq, 09 Jul 2006	*KJ162095
"P." inamoena clade	K202	Louisiana-Texas Plateau, USA	S. Fredericq, 09 Jul 2006	EU349124
Riquetophycus clade	K204	Louisiana-Texas Plateau, USA	S. Fredericq, 09 Jul 2006	EU349156
"P." inamoena clade	K228	Texas Plateau, USA	S. Fredericq, 07 Sep 2003	EU349133
Riquetophycus clade	LAF277	Louisiana-Texas Plateau, USA	S. Fredericq, 01 Jul 2001	EU349159
Riquetophycus clade	LAF3901	Louisiana-Texas Plateau, USA	S. Fredericq, 02 Dec 2010	*KJ162102
Riquetophycus clade	LAF3902	Louisiana-Texas Plateau, USA	S. Fredericq, 02 Dec 2010	*KJ162103
Riquetophycus clade	LAF3903	Louisiana-Texas Plateau, USA	S. Fredericq, 02 Dec 2010	*KJ162104
Riquetophycus clade	LAF3908	Louisiana-Texas Plateau, USA	S. Fredericq, 02 Dec 2010	*KJ162106
Riquetophycus clade	LAF6580	Louisiana-Texas Plateau, USA	S. Fredericq 26 Aug 2012	*KJ162105
"P." inamoena clade	LAF6581	Louisiana-Texas Plateau, USA	S. Fredericq 26 Aug 2012	*KJ162097
New Genus Clade	LAF6582	Louisiana-Texas Plateau, USA	S. Fredericq 26 Aug 2012	*KJ162098
New Genus Clade	LAF6583	Louisiana-Texas Plateau, USA	S. Fredericq 26 Aug 2012	*KJ162100
New Genus Clade	LAF6584	Louisiana-Texas Plateau, USA	S. Fredericq 26 Aug 2012	*KJ162099
"*Rhizophyllis pacifica*"	LAF6585	Louisiana-Texas Plateau, USA	S. Fredericq 26 Aug 2012	*KJ162090
"*Contarinia magdae*"	LAF6586	Louisiana-Texas Plateau, USA	S. Fredericq 26 Aug 2012	*KJ162091
"*Contarinia magdae*"	LAF6587	Louisiana-Texas Plateau, USA	S. Fredericq 26 Aug 2012	*KJ162092
Nesophila hoggardii W. A Nelson *et* N. M. Adams				EU349210
Ochtodes secundiramea (Montagne) M. A. Howe				EU349209
Portieria japonica (Harvey) P. C. Silva				U26825
Portieria hornemannii (Lyngbye) P. C. Silva				EU349206
Portieria sp.				AF212185
Portieria tripinnata (Hering) De Clerck				EU349205
Rhizophyllis squamariae (Meneghini) Kützing				EU349208
Kallymenia cribrosa Harvey				EU349216
Callophyllis pinnata Setchell *et* Swezy				AY294397
Chondrus crispus Stackhouse				U02984
Contarinia okamurae Segawa				EU349088
Dilsea californica (J. Agardh) Kuntze				U04192
Dumontia contorta (S. G. Gmelin) Ruprecht				AY294378
Gigartina pistillata (S. G. Gmel.) Stackhouse				AY294371

RESULTS

Our observations from the six post-spill sampling expeditions centered on previously studied hard banks west of the mouth of the Mississippi River and extending east to offshore Alabama, an area encompassing roughly 27°58'N to 29°26'N and 87°34'W to 91°01'W, indicate that seaweed diversity had dramatically declined (Figs 9-10), changed, and were all together absent (Figs 8, 11-12) at dredged sites at Ewing Bank and Sackett Bank (Fig. 7) relative to pre-spill sampling at the same sites and depths (Figs 2-3) (pers. obs.). Ewing Bank used to
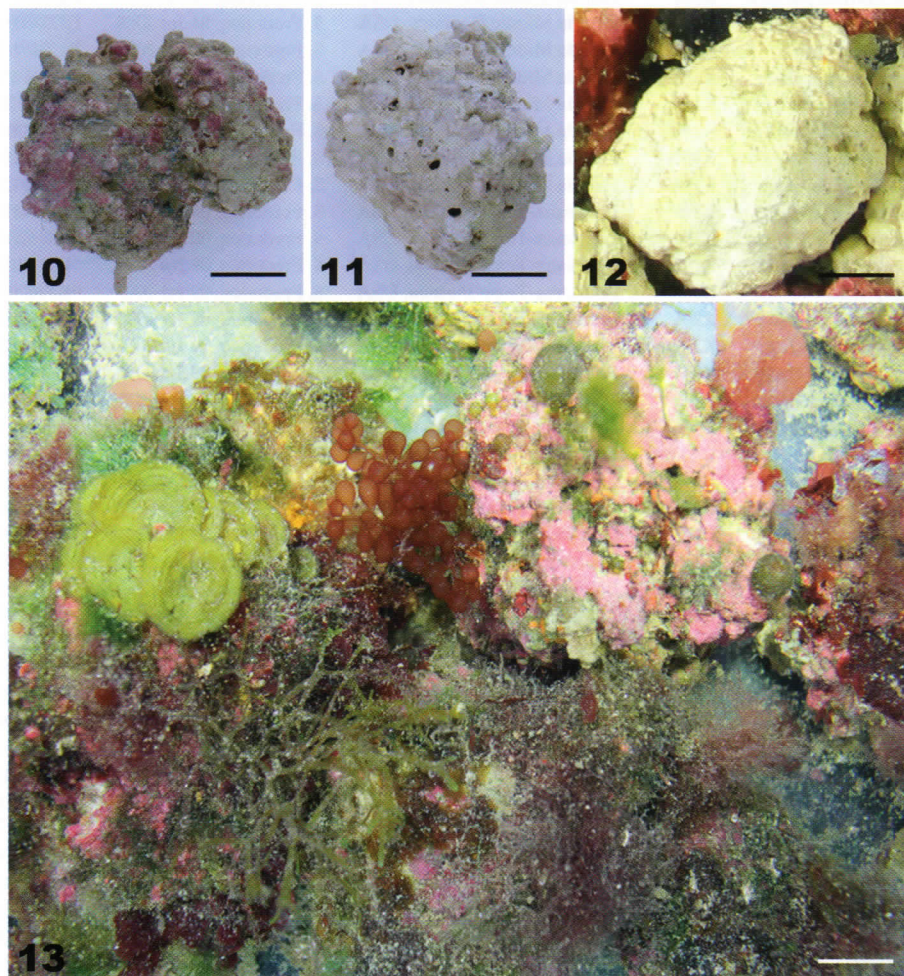

Figs 10-13. **10.** Partly algal-denuded rhodolith from Ewing Bank, Aug. 2011, ~65 m depth. Scale = 1 cm. **11.** Bleached rhodolith from Ewing Bank, Aug. 2011, ~65 m depth, Scale = 1 cm. **12.** Bare, autogenic rubble collected from Ewing Bank at 55 m depth, coll. 8/29/2011, placed in microcosm tank. Photo taken on 9/4/2011. Scale = 1 cm. **13.** Microcosm showing lush and vital assemblage of green, red and brown algae collected from partly denuded rubble at Ewing Bank 2 on 8/29/2011. Photo taken on 10/6/2011. Scale = 1.5 cm

harbor one of the northwestern Gulf's most diversity-rich seaweed communities in terms of number of species (Fredericq *et al.*, 2009) and algal biomass prior to the DWH oil spill (pers. obs.). Nevertheless, most of the rhodoliths dredged in Ewing Bank looked chemically "bleached" (Figs 10, 11) post-spill, or if crustose taxa were alive, rhodoliths were devoid of fleshy, conspicuous macroalgae (*i.e.* "algal-denuded"). The few seaweed species we collected in our first post-spill expedition
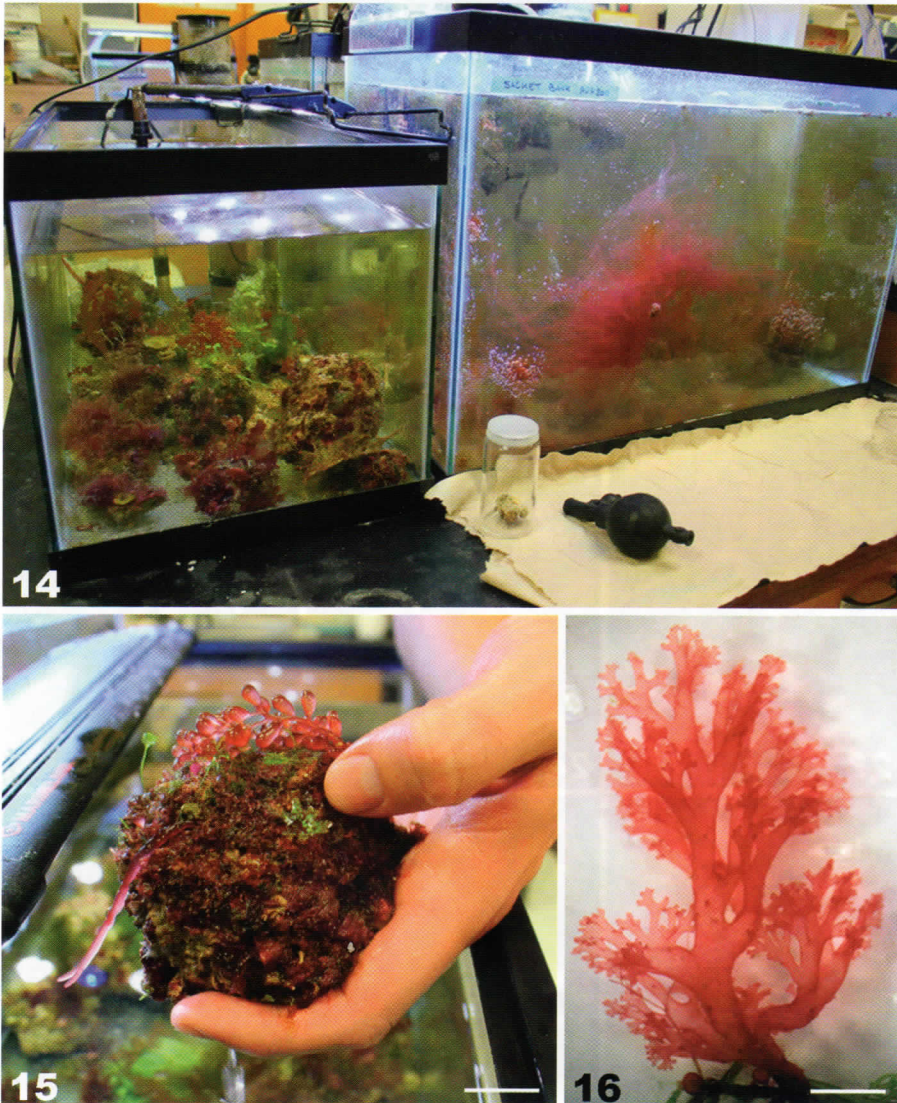
Figs 14-16. **14.** Luxurious growth of rhodoliths covered by macroalgae in microcosms. Algal-denuded rhodoliths were collected from Ewing Bank, 08/11 (left) and bare rubble from Sackett Bank (right). Photo taken on 2/16/2012. **15.** Live rhodolith from microcosm in Fig. 14 photographed on 2/16/12 bearing luxurious growth of macroalgae. Scale = 2 cm. **16.** Female gametophyte of *Schmitzia* sp. growing from bare rubble collected from Ewing Bank in a 8/11 microcosm. Photo taken on 5/2012. Scale = 0.2 cm.
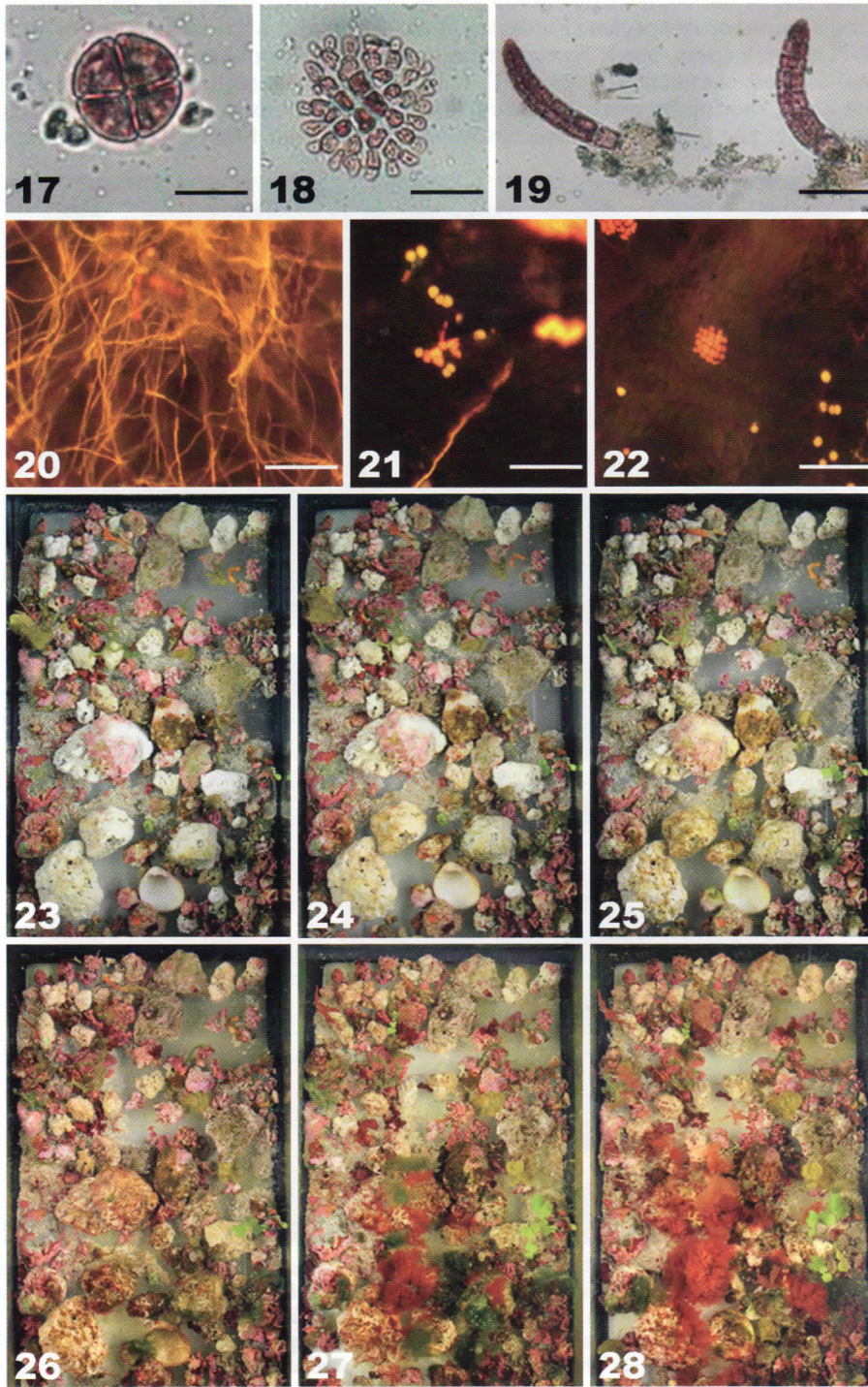
(Dec. 2010) included predominantly green gelatinous algae (*Verdigellas*) and a few crustose corallines and/or red algal crusts of Peyssonneliaceae (Peyssonneliales). Upon their transport to the laboratory and incubation in ~75 Liter controlled microcosms, these bare or partly algal-denuded unconsolidated rubble were maintained in ~75 L salt-water microcosms in our laboratory. Within a three-week time frame, the "bare" rocks (Fig. 12) placed in the seawater tanks gradually became covered by a suite of red, green and brown seaweed germlings that to this day continue to grow to adult size (Figs 13-15, 23-28) revealing vital biodiversity apparently repressed in the NWGMx at the time of post-spill sampling since the Deepwater Horizon oil spill. Furthermore, several algal species currently growing and reproducing in our tanks, such as *Schmitzia* sp. (Fig. 16) had not previously been observed in the field pre-and post-spill. Biweekly digital photography of the rhodoliths within each microcosm documents a remarkably fast rate of algal succession (Figs 23-28), with germlings being visible to the naked eye within two-three weeks and reaching maturity at ~10 weeks. The molecular and morphological characterization to resolve the taxonomic identity of the biodiversity found in these microcosms is ongoing. We cannot provide rigorous quantitative data regarding rates of algal recovery because of lack of sufficient replication, and haphazardous and qualitative collecting devices in the field.

Ten-day old red algal propagules and germlings, green algal spores and bacteria readily settled on glass microscope slides (Figs 17-19) immersed in our August 2011 Ewing Bank microcosm. Images obtained from epifluorescence microscopy on rhodolith samples from the Aug. 2011 Ewing Bank microcosm clearly show the presence of endolithic (Fig. 20) red algal filaments and siphonous green algae (e.g. *Ostreobium* sp.) and epilithic red algal germlings and green algal spores on the surface of the same piece of rubble (Figs 21-22).

Prior to the Deepwater Horizon oil spill in the NW Gulf of Mexico members of the crust-forming Peyssonneliaceae (Peyssonneliales) (Fig. 29) and Rhizophyllidaceae (Fig. 30) were the principal rhodolith-encrusting taxa. Their molecular characterization, on the basis of comparative chloroplast-encoded *rbc*L sequence analysis revealed 26 distinct rhodolith-dominating species of Peyssonneliaceae for the entire Gulf of Mexico (Fig. 29), of which 9 had been documented in the NWGMx before the DWH oil spill (Fig. 29, taxa highlighted in blue) and 5 distinct species were present in the NWGMX subsequent to the oil spill (Fig. 29, taxa highlighted in pink). Interestingly, common pre-spill NWGMx taxa, such as "*P. inamoena*" (top clade in Fig. 29) have not been recollected post-spill. A southeastern Gulf species ("New genus clade" in Fig. 29) was never collected pre-spill in the northwestern Gulf but well post-spill. Finally, a species of *Riquetophycus* (in "*Riquetophycus* clade" in Fig. 29) has consistently been as dominant before as after the DWH oil spill.

◄ Figs 17-28. Ten-day old settled red algal propagules and germlings, green algal spores and bacteria settled on glass microscope slides from Aug. 2011 Ewing Bank microcosm tank. Photos March 1, 2012. Fig. 17 Scale = 16 μm, Fig. 18 Scale = 24 μm, Fig. 19 Scale = 38 μm. **20-22.** Epifluorescence microscopy images showing presence of endolithic red algae and propagules (shown in orange) and siphonous green algae (shown in yellow, e.g. *Ostreobium* sp.) (Fig. 20) inside piece of rubble from Aug. 2011 Ewing Bank microcosm, and epilithic red algal germlings and green algal spores on surface of same piece of rubble, Figs 21-22). Photos taken on 2 March 2012. Scale = 80 μm. **23-28.** Microcosm showing rhodoliths with algal succession photographed at biweekly intervals. Rhodoliths initially dredged as denuded or bare rubble at Ewing Bank on 8/12/2012, 70 m depth.
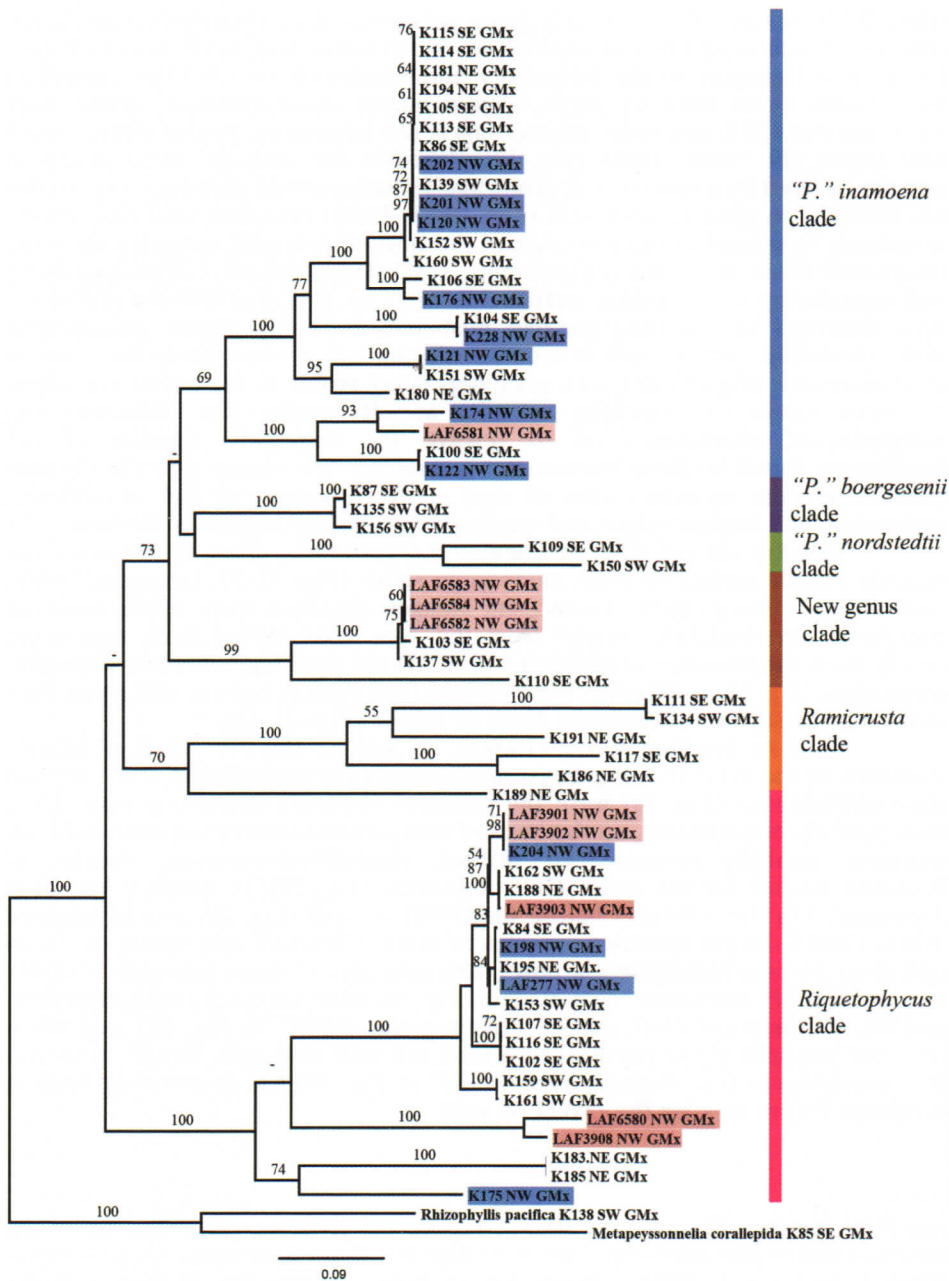
Fig. 29. *Rbc*L tree of 62 members of Peyssonneliaceae (Peyssonneliales) associated with rhodoliths in the Gulf of Mexico (northwestern = NW, southwestern = SW, northeastern = NE, southeastern = SE representing 29 species and 6 distinct genera. Highlighted in blue are northwestern Gulf taxa collected before the DWH oil spill; highlighted in pink represents NW taxa collected after the DW oil spill. Outgroup taxa are *Rhizophyllis pacifica* and *Metapeyssonnelia corallipeda*, Rhizophyllidaceae, Dumontiaceae-complex.
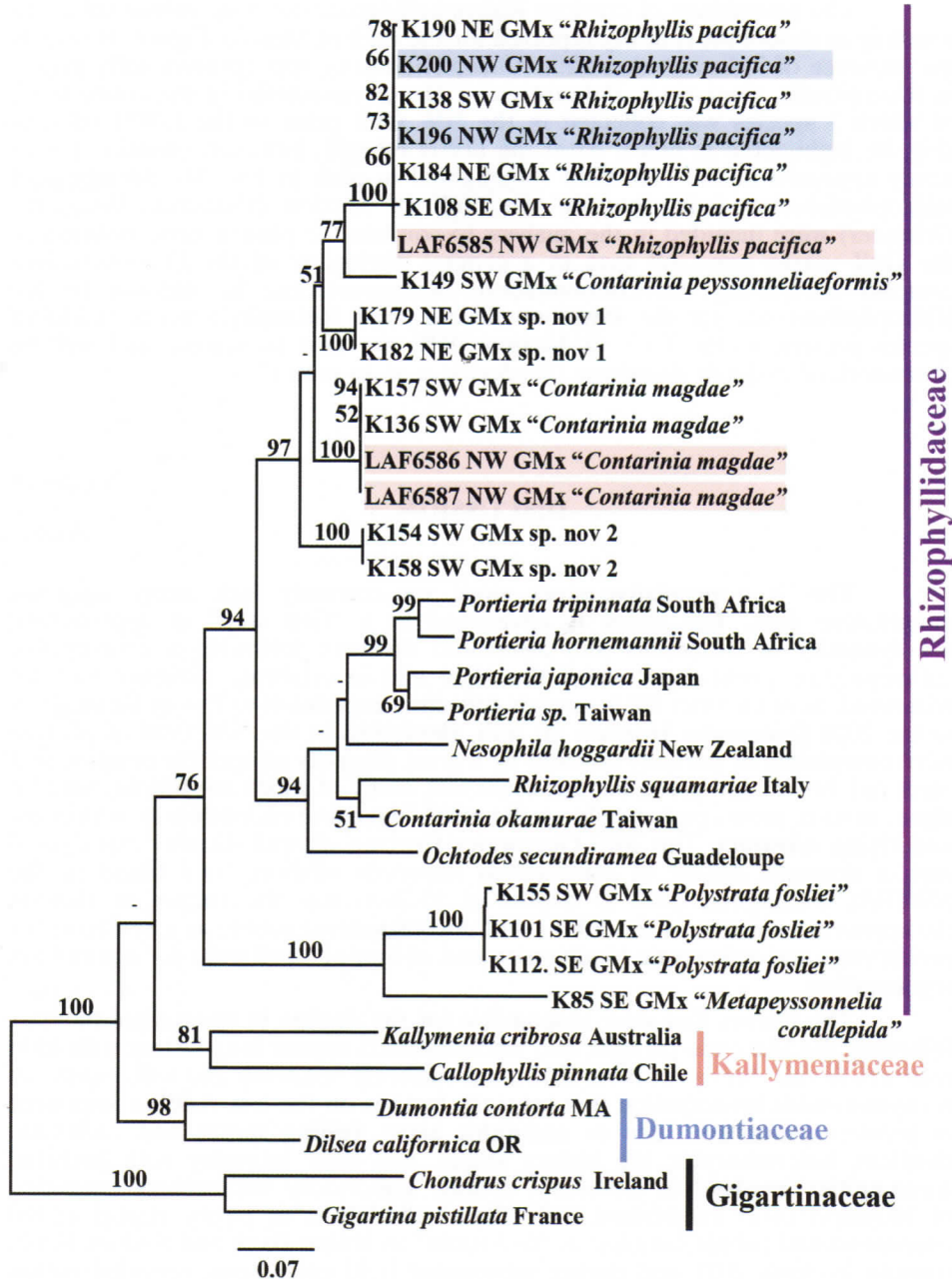
Fig. 30. *Rbc*L tree of the Dumontiaceae-complex representing 28 members of Rhizophyllidaceae, 2 species of Dumontiaceae and 2 species of Kallymeniaceae. Associated with rhodoliths in the Gulf of Mexico (northwestern = NW, southwestern = SW, northeastern = NE, southeastern = SE are 19 members of Rhizophyllidaceae representing 7 species and 3 distinct genera. Highlighted in blue is a NW Gulf taxon collected before the DWH oil spill; highlighted in pink represents NW taxa collected after the DW oil spill.

The assemblage of crustose Rhizophyllidaceae covering rubble substrata resulting in rhodoliths is newly reported for the Gulf of Mexico. Figure 30 reveals the presence of 7 distinct rhodolith Rhizophyllidaceae spp. (provisionally placed in *Rhizophyllis, Contarinia, Polystrata* and *Metapeyssonnelia*) in the entire Gulf, of which 1 species was collected in the NW Gulf prior to the DWH oil spill (Fig. 30, highlighted in blue). After the DWH oil spill, however, another species newly appeared in the NW Gulf (highlighted in pink in Fig. 34). Members of Rhizophyllidaceae living outside the Gulf of Mexico (*Portieria, Nesophila, Ochtodes*) were included in the analysis to confirm the phylogenetic position of the Gulf versus non-Gulf taxa in a general phylogeny of the Dumontiaceae-complex comprising the Dumontiaceae, Kallymeniaceae in addition to the Rhizophyllidaceae. Of the Peyssonneliaceae- and Rhizophyllidaceae-rhodolith species present in the Gulf of Mexico, 94% are new to science and will be characterized in detail elsewhere (Fredericq *et al.* in prep.).

DISCUSSION

This is a speculative paper since we currently lack many rigorous, quantitative data. The paper is envisioned as a "first step" in approaching the dynamics of rhodoliths and associated diversity following a catastrophic anthropogenic event from which the algal and invertebrate diversity has not recovered, as of October 2013, our last collecting expedition to Ewing Bank. Prior to the 2010 Deepwater Horizon oil spill, rhodoliths in the NW Gulf of Mexico were completely or partially covered by a great diversity of epilithic crustose and erect red, brown and green benthic seaweeds, many of which are foliose. Smaller algae, in turn, grew epiphytically on the macroalgae and endolithically within the underlying substrata. The 2010 Deepwater Horizon oil spill disaster that caused such a dramatic die-off of conspicuous seaweeds offshore hard banks in the NWGMx continental shelf at depths of 55-70 m was the trigger to viewing the apparent vital role of rhodoliths and unconsolidated rubble as a reservoir for prokaryotic and eukaryotic biodiversity and as marine seedbanks for community regeneration.

The factors that were responsible for the decline in macroalgal biomass following the Macondo oil spill, and whose impacts appear long-lasting with little recovery to this day (as of Nov. 2012), are currently unknown and will require an ecosystem-wide investigation that specially focuses on the less visible component of primary producers, such as endolithic algae, resting spores and carbonate dwellers, heteromorphic life history stages, and their interplay with bacterial communities involved in elemental cycling. Laboratory microcosms consisting of 20-gallon tanks established with "bare" rhodoliths or partly algal-denuded unconsolidated rubble sampled as "live rocks" at Ewing Bank and Sackett Bank, starting in April 2011 and during subsequent field collections, revealed within three weeks a wealth of red, green and brown seaweed germlings that to this day continue to grow to adult size, reproduce and go through a series of dynamic successions including temporal biomass decline and subsequent regrowth in our tanks. Algal biodiversity in each microcosm reflects the same species composition that was present in the field before the DWH oil spill, suggesting that germination of resting spores in the field may currently be repressed by unknown factors.

Seaweed propagules, germlings, and bacteria settled on glass slides from the ambient seawater in were visible within (endolithic) and/or on top (epilithic) of rubble substratum through epifluorescence microscopy, indicating that alternative resting stages of algae were already present within the rhodoliths at the time of their post-spill sampling. Furthermore, many of these algal species currently growing and reproducing in our tanks, such as an undescribed species of *Schmitzia* (Calosiphoniaceae), had not been observed in the field during our pre-spill sampling, perhaps due to their rare or ephemeral nature.

One of the most fundamental and precarious stages in the life history of a benthic alga is the colonization of a new substratum (Hoffmann & Santelices, 1991; Maggs & Cheney, 1990; Fletcher & Callow, 1992; Amsler *et al.*, 1992; Kaehler & Williams, 1997; Ista *et al.*, 2004) for their life cycle completion, persistence and development (Richardson, 1981). Calcium carbonate substrata are well-known to be essential in the life cycle of some seaweeds in which the heteromorphic stage of the sporophyte individual (in which meiosis occurs) is often a small crust, disk or aggregation of creeping filaments that does not resemble the larger gametophyte individual (Hommersand & Fredericq, 1990; Guiry, 1990; Hawkes, 1990). Although there currently is only anecdotal evidence on viewing a major function of deepwater rhodoliths in the offshore hard banks of the Gulf of Mexico as possible banks of dormant stages of benthic seaweeds, it has been postulated that the dynamics of a intertidal banks of settled algal spores germinating into microscopic forms and those of land plant seedbanks are analogous in several ways, e.g., remaining in the soil until environmental stimuli trigger germination (Hoffmann & Santelices, 1991). Such banks of microscopic algal stages may be subjected to similar interactions with bacterial or fungal communities as land plant seeds, such as predation or facilitation (Hoffmann & Santelices, 1991).

Since the post-spill collected rhodoliths were placed in microcosms containing seawater that was retrieved *in situ* (*i.e.* at the same site and same depths), algal spores and microbionts could only have come along with the rhodoliths at the time of sampling or ambient seawater. Louisiana light crude oil that leaked from the Macondo Well is composed of hydrocarbons and other organic compounds containing nitrogen, sulfur, and metals (e.g. iron, nickel, copper) that may have stimulated components of the bacterial communities to the detriment of the algae. One potential hypothesis for this pattern is that introduction of such oil into porous bedrock may have stimulated (and continue to stimulate) anaerobic sulfate reducers leading to the production of hydrogen sulfide. When dissolved in water, H_2S becomes hydrosulfuric acid, a weak acid whose corrosive effects may explain why so many post-spill rhodoliths upon contact with the acid were stripped of their surface layers or look chemically "bleached." It is possible that under these scorching conditions the conspicuous vegetative, macroalgal thallus component of a seaweed's life cycle may have died, leaving on-site only a refugium of resting stages (spores, propagules) alive within a rhodolith, awaiting more favorable environmental conditions to germinate. We hypothesize that once the rhodoliths were removed from the deep banks' sulfide habitat and placed in our microcosms, the algal spores/microbionts contained within/associated with the rhodoliths were triggered to resume their metabolic activities and germinate, grow and reproduce in our microcosms. In contrast, heavy fractions of oil may have been retained in the sediments underlying the rubble habitats and may continue to seep sulfide with long-term negative impacts on macroalgal assemblages and higher trophic levels that depend on them as a resource or habitat. We have continuously been sampling in these offshore hard

banks since 1996, and before the DWH oil spill the rhodoliths were never bleached as they were post-spill. We did not change our sampling technique following the oil spill and the oil spill would not have caused the burial of the rhodoliths. Post-spill videoclips of the rhodolith beds *in situ* did not reveal any buried rhodoliths, and most were bleached and bare (see series of videoclips on http://youtube.com/nemastoma2). The reported chemical bleaching is a hypothesis that still needs to be tested.

Another oil-induced stress on the rhodolith algal community following the DWH oil spill could have been an increase in their release of dimethylsulfoniopropionate (DMSP) production, a sulfur-containing molecule that is metabolized by anaerobic prokaryotes, and may also have contributed to sulfide production, further stressing the remaining macroalgal standing stock in the field. Coralline algae, among other primary producers, directly affect the global sulfur cycle and global climate change (Gabric *et al.*, 1998), and are considered to be one of the major DMSP producers (Kamenos *et al.*, 2008), which, upon being metabolized by bacteria associated with the algae, produces volatile compounds such as dimethyl sulfide (DMS) (Wilhelm *et al.*, 1997), ultimately released to the atmosphere, where it plays a role in increasing its albedo. DMSP may also be a grazing deterrent (e.g. Sunda *et al.*, 2002).

Whether prokaryotes and algal communities act independently or synergistically in the NWGMx to promote environmental health, ecosystem stability, productivity, resilience, or biological adaptation in response to rapid environmental change, such as the DWH oil spill, is currently poorly understood. Do specific endolithic algal communities in NWGMx rhodoliths drive specific bacterial communities, or vice-versa? Is there a functional analogy between crustose coralline algae growing in coral reefs where they generally encourage benign microbial communities while turf algae and fleshy macroalgae promote heterotrophic microbial diversity (Barott *et al.*, 2012), and rhodoliths growing in the offshore banks of the NWGMx? Since algae release chemically diverse organic carbon in their direct environment (e.g. Smith *et al.*, 2006) that is utilized by co-habiting prokaryotes, do the latter in turn cycle key biogeochemical elements necessary to these primary producers and other rhodolith colonizers? These interesting points may have many implications for better understanding important ecological cues, such as spore settlement in rhodolith beds of the NWGMx and worldwide.

The pathways in which bacteria interact with eukaryotic algae are extremely diverse and may either suppress or trigger their germination (e.g. Dillon, 1989; Egan *et al.*, 2001; Mayali & Doucette, 2002; Callow *et al.*, 2003; Matsuo *et al.*, 2003, 2005; Dobretsov *et al.*, 2006; Weinberger *et al.*, 2007; Barott *et al.*, 2011; Barott & Rowher, 2012). However, nothing is currently known about microbial effects on Gulf of Mexico associated-rhodoliths in order to understand the dynamics of various algal/bacterial assemblages in the NWGMx and how they relate to the function of rhodolith beds for ecosystem resilience and regeneration (*i.e.* our "seedbank" hypothesis). Another substantial gap in biodiversity knowledge is assessing the potential cyanobacterial community growing associated with the rhodoliths that might also facilitate nodule calcification and cementation, implicating a potential symbiotic association (Kazmierczak & Iryu, 1999).

CaCO$_3$ is precipitated extracellularly as calcite in coralline-dominated rhodoliths and as aragonite in most non-coralline rhodoliths (Lobban & Harrison, 1994; Lee & Carpenter, 2001). Commonly found penetrating calcareous substrata in general are endolithic marine fungi (Golubic *et al.*, 2005), cyanobacteria (Zhang & Golubic, 1987) and siphonous green algae (Tribollet & Payri, 2001). Research

on carbonate components of relict continental margin sediments typically focuses on the negative effects of endolithic microborers (e.g. Golubic, 1969; Perkins & Halsey, 1971; Lukas & Golubic, 1983; Macintyre et al.. 2000) and endolithic algae (Fine et al., 2006; Gutner-Hoch & Fine, 2011) and fungi (Yarden et al., 2007) on hermatypic corals (Wisshak et al., 2005) as agents of substratum erosion (Tribollet et al., 2002, Tribollet & Golubic, 2005) or pathogenic agents (Yarden et al., 2007; Bentis et al., 2000). Previous studies on endolithic microborer communities have shown that the arrangement of the endolithic biota in carbonate substrata is dynamic (Wisshak et al., 2005) and can shift due to a decrease in seawater pH (Tribollet et al., 2009), or alteration in organic material available (Carreiro-Silva et al., 2009) in the ambient water. Since rhodoliths are well known as important "ecosystem engineers" (Foster et al., 2007; Pacheco, 2010) providing a structurally complex habitat associated with many microhabitats (Peña et al., 2008; Pacheco, 2010) for diverse assemblages of invertebrates, micro- and macroalgae and other taxa (Cabioch, 1969; Kegan, 1974; Bosence, 1983; Steller et al., 2003; Hinojosa-Arango et al., 2004), we hypothesize that the rhodoliths may further engineer ecosystem resilience and regeneration through cryptobiosis or seedbank dormancy during environmental stress as well. This, combined with a complex food web that derives energy from a diversity of carbon sources, may help explain the high number of species in this exceptional ecosystem (Barbera et al., 2003; Grall et al., 2006). Since crustose corallines in general are known to release chemosensory compounds that have been implicated in the larval settlement and morphogenesis of a range of invertebrates, especially reef-building corals (Morse et al., 1996; Heyward & Negri, 1999; Hadfield & Paul, 2001; Harrington et al., 2004), mollusks (Roberts, 2001), cnidarians, crustaceans, echinoderms (Riosmena-Rodríguez & Medina López, 2011), sponges (Avila & Riosmena-Rodríguez, 2011), and gorgonians (Georgiadis et al., 2009), coralline-dominated rhodolith beds in the NWGMx may likewise be implicated in controlling the dynamics of their microbionts as well.

Better baseline data on rhodolith algal species composition and its associated microbiont biodiversity in relation to elemental biogeochemical cycling are sorely needed to better understand their ecosystem functioning and ecosystem-level consequences of human-mediated disturbances such as oil spills. A great deal more work is required to fully understand the ecosystem services that the rhodolith nodules provide (Scherner et al., 2010), and the roles and importance of calcified algae and maerl in global carbon processes and sulfur processes. Moreover, bacterial communities in/on rhodoliths may play an important metabolic role in nitrogen and phosphate cycling, two important chemical elements fueling primary production. For example, the aquarium trade retails large amounts of "live rocks" (basically rhodoliths collected on reef slopes, i.e. 2 million pound imported in 2006 and 500,000 pounds in 2008 Rhyne et al., 2012) for the natural cycling of nutrients, and for organism "seeding" to establish functional coral reef microcosms (Delbeek & Sprung, 2005). Responding to a popular audience of hobbyists eager to maintain long-term vital and healthy saltwater aquaria, Delbeek (1994) knew from experience that "For the aquarium trade this rock is highly valued not only for the diversity of life it can bring to the closed marine environment, but its function as a superior biological filter that hosts both aerobic and anaerobic nitrifying bacteria required for the nitrogen cycle that processes waste. Live rock becomes the main biological nitrification base or biological filter of a saltwater aquarium. Additionally, live rocks have a stabilizing effect on the water chemistry, in particular on helping to maintain constant pH by release of calcium carbonate."

Autogenically derived rhodoliths, *i.e.* unconsolidated calcium carbonate rubble established by differential erosion processes of diapir salt in the NW Gulf of Mexico, are for the most part dominated by Peyssonneliaceae in the Gulf of Mexico; a well-supported molecular-based phylogeny recognizes twenty-six distinct species spread over six genera in the Gulf alone. Less common are Rhizophyllidaceae-dominated rhodoliths, with the new recognition of six distinct species for the region spanning 6 genera. This extraordinary and previously unrecognized, hidden macro-biodiversity encompassing a rhodolith points to the amount of taxonomic, phylogenetic, and biogeographic research that still needs to be done in the Gulf habitats and worldwide. Scanning electron microscopy data are needed to compare the rhodoliths' geochemistry origin and nucleation process, the complex micro-anatomy of its surface, and its internal structure composition at various depths on samples collected before and after the DWH oil spill, as well as in-depth elemental analysis and chemical characterization using Energy Dispersive X-ray spectroscopy (SEM-EDS).

To assess the causes for overall community dynamics and resilience, research is urgently needed on in-depth sequencing of eukaryotic and bacterial rhodolith-habitat community DNA (environmental sequencing) and community RNA (metatranscriptomics) for assessing the metabolic processes taking place in these rhodolith/hard bank rubble communities. Such approach will require the use of Next-Generation Sequencing (NGS) tools that have revolutionized microbial ecology in recent years and accelerated diversity discovery (e.g. DeLong, 2009). Viewing rhodoliths and unconsolidated rubble in the offshore NWGMx as a functional unit for element cycling and establishment of dynamic marine seedbanks and associated cryptic biodiversity is vital to understanding species-specific responses in case of disturbances (see Nelson, 2009). More than ever, it is especially timely to define the essential ecological functional dynamics of rhodoliths beds in the northwestern Gulf of Mexico from an algal/bacterial perspective since it relates to the recent disappearance of conspicuous (visible) macroalgae in the hard banks in proximity of the Macondo well following its blowout and oil spill insult.

CONCLUSION

Based on 6 post-spill expeditions to offshore deep hard banks in the NWGMx, there does not seem to be any significant algal recovery in the field following the DWH oil spill. Our research is still in the observational and exploratory stage and we cannot provide rigorous quantitative data regarding rates of algal recovery in laboratory microcosm experiments because of lack of sufficient replication. We newly hypothesize that rhodoliths serve as "seedbanks" for biological diversity since apparently "dead" rubble collected in the field became regenerated by epi- and endolithic algae and microbes in microcosm settings. Decreases in seaweed abundance in the field may be linked to a shift in nutrient availability and various microbial connections, to be tested with further research.

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