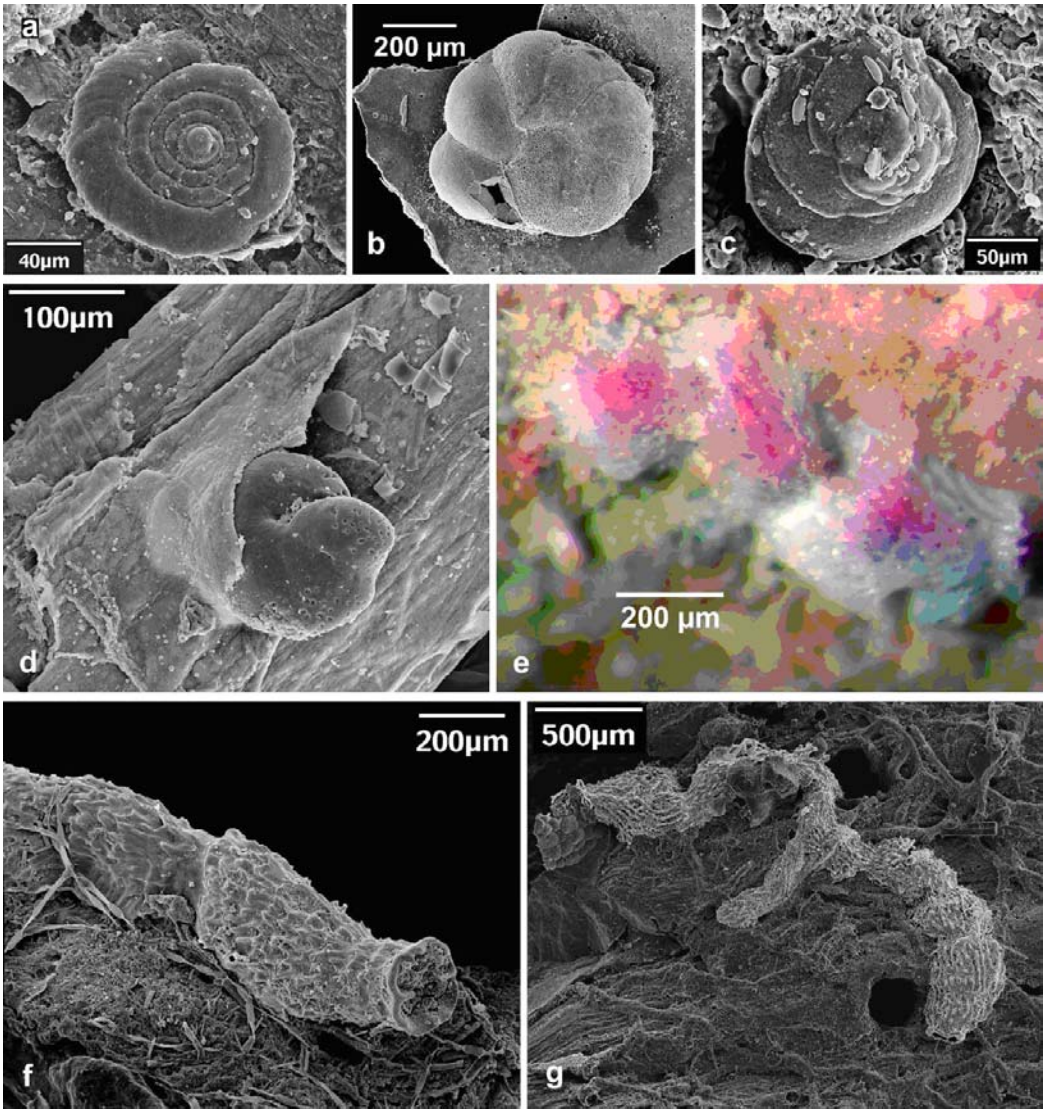
OCS Study
MMS 2009-059

Coastal Marine Institute

Evaluation of Oil and Gas Platforms on the Louisiana Continental Shelf for Organisms with Biotechnology Potential



MMS U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

LSU Cooperative Agreement
Coastal Marine Institute
Louisiana State University

OCS Study
MMS 2009-059

Coastal Marine Institute

Evaluation of Oil and Gas Platforms on the Louisiana Continental Shelf for Organisms with Biotechnology Potential

Editor

L. Rouse

November 2009

Prepared under MMS Cooperative Agreement
1435-01-99-CA-30951-17809
by
Louisiana State University
Coastal Marine Institute
Baton Rouge, Louisiana 70803

Published by

U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

Cooperative Agreement
Coastal Marine Institute
Louisiana State University

DISCLAIMER

This report was prepared under contract between the Minerals Management Service (MMS) and Louisiana State University. This report has been technically reviewed by the MMS, and it has been approved for publication. Approval does not signify that the contents necessarily reflect the view and policies of the MMS, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. It is, however, exempt from review and compliance with MMS editorial standards.

REPORT AVAILABILITY

This report is available only in compact disc format from the Minerals Management Service, Gulf of Mexico OCS Region, at a charge of $15.00, by referencing OCS Study MMS 2009-059. The report may be downloaded from the MMS website through the Environmental Studies Program Information System (ESPIS). This report can also be obtained from the National Technical Information Service. The addresses are below. Copies can also be inspected at selected Federal Depository Libraries.

Minerals Management Service
Gulf of Mexico OCS Region
Public Information Office (MS 5034)
1201 Elmwood Park Boulevard
New Orleans, Louisiana 70123-2394
Telephone requests may be placed at
(504) 736-2519, 1-800-200-GULF, or
Rush Orders: 1-800-553-6847
Fax: (504) 736-2620

U.S. Department of Commerce
National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161
Phone: (703) 605-6040
Fax: (703) 605-6900
Email: bookstore@ntis.gov

CITATION

Rouse, L., ed. 2009. Evaluation of oil and gas platforms on the Louisiana continental shelf for organisms with biotechnology potential. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2009-059. xi + 53 pp.

ABOUT THE COVER

Day and night images of the semisubmersible petroleum platform “Nakika” located in the Gulf of Mexico at Mississippi Canyon Block 474. Images by David L. Nieland, Coastal Fisheries Institute, Louisiana State University, Baton Rouge, Louisiana.

PROJECT OVERVIEW

This project was developed in response to MMS's recognition that offshore oil and gas platforms may serve as a harvestable source of organisms with pharmaceutical or other commercial applications. The first concern of this study, therefore, was, to identify the organisms that make up the biofouling or epifaunal communities on platforms. The second objective of this study was to preliminarily determine the potential of any of these organisms to be source of pharmaceuticals or any other commercially important products. Finally, the concentration and distribution of potentially important species on the structures and the variability of that distribution with platform location, and the density of organisms at various depths on the platform and seasonal variation of occurrence was considered.

Some of the groups of organisms common to the Gulf of Mexico platforms that show potential for biotechnology applications were included in this study. These include bacteria, particularly members of the Class Actinobacteria, that have yielded numerous bioactive compounds valued as a source of pharmaceuticals and enzymes; marine algae that provide a range of natural products from agar to pharmaceuticals; benthic foraminiferans, especially agglutinated wall species; byssate molluscs that have potential in the production of bioadhesives for many uses from transdermal drug delivery systems to surgical adhesives; and bryozoans, particularly *Bugula neritina,* the only source of bryostatin, a drug used in cancer treatment.

Two collecting trips were made from Port Fourchon, Louisiana, on the M/V *Spree*. On the first cruise, June 9, 2001, Tropical Storm Allison prevented sampling except at one of the five chosen platforms, ST 23. On the second cruise, May 4-5, 2002, four platforms were sampled and one platform could not be sampled due to dangerous diving conditions.

At each sampling site, divers outlined a 25 cm x 25 cm area, using a metal template. A diver suctioned loose surface material from this area with a 60 cc syringe. All encrusting organisms were then scraped and preserved by methods appropriate to the taxa studied. The following is a summary of the findings from each group of organisms.

BACTERIA

The study demonstrated that the biomatrix associated with gas and oil platforms contains large numbers of bacterial species many of which are novel and belong to taxonomic groups known to be of biotechnological importance.

The construction of total genome libraries from the biomatrix of oil and gas platforms and their expression will no doubt provide novel bioactivities from both the micro and macro components of these complex systems. This study established that the biomatrix encrusted on oil and gas platforms represents a potential starting material for such studies.

ALGAE

The total number of algal taxa collected during this study was 24. The best represented group was Rhodophyta (approximately 50% of the taxa collected). Seven new taxa were added to the list of macroalgae identified from the platforms and one new report of *Antithamnionella breviramosa* from the Gulf of Mexico. Approximately 50% of the taxa collected are known to have biotechnological potential.

FORAMINIFERA

A rich assemblage of benthic Foraminifera was recognized in the scrapings and the syringe samples. The species included both grazers and attached forms. The assemblage density was highly variable.

The most conspicuous aspect of the foraminiferal distribution was the abundance of attached taxa; some sessile species that are rare or absent in soft substrates live in relative profusion on the platforms. In addition, the foraminiferal community includes many vagrant foraminiferal species. Agglutinated foraminiferal species hold promise as a source of bioadhesives for biotechnological and biomedical applications, because they can secrete and then harden adhesive organic compounds in an aqueous medium although no species with known biotechnical potential were found.

BRYOZOA

To date the only marine compound to enter phase II clinical trials is bryostatin 1. Bryostatin 1 combats the growth of cultured cancer cells and has shown some promise aiding patients suffering from non-Hodgkin's lymphoma and lymphocytic leukemia. Bryostatin 1 was initially isolated from the bryozoan *Bugula neritina*, but in California that named species turned out to be composed of several cryptic species, only one of which produces bryostatin I. The compound occurs in an endosymbiotic bacteria present within *B. neritina*.

B. neritina as currently recognized in US waters comprises three cryptic species: the deep water Pacific form, a Shallow/Southern form present both in shallower Pacific waters and along the Atlantic coast south of Cape Hatteras, and a third form present in the Atlantic north of the Cape Hatteras region. This study found that neither the species collected from the Gulf of Mexico platforms nor those found in the Atlantic produce the commercially important bryostatin I.

MOLLUSCS

The platforms sampled hosted a low diversity molluscan assemblage dominated by byssate bivalves. Twenty-seven bivalve and gastropod species were identified, with *Isognomon bicolor* and *Barbatia candida* making up about 90% of the total assemblage. Changes in relative abundance of taxa among platforms were in part caused by mollusks collected in this study with the greatest biotechnology potential include the byssate bivalves *Isognomon* and *Barbatia* that produce bioadhesives. The byssus is a bundle of proteinaceous threads secreted by some bivalves that attaches to a substrate by an adhesive plaque. These adhesives are of biotechnological interest because they provide strong, durable adhesion to wet surfaces.

Taxonomic and genetic heterogeneity of marine organisms must be anticipated prior to surveys for biotechnologically useful molecules and studies of ecological processes. This study examined the genetic variability of the bivalve community associated with northern GOM oil platforms in order to (1) determine if there were cryptic species and (2) examine the genetic variation within and among platforms. Data collected indicated that all of the individuals were *Barbatia candida*.

TABLE OF CONTENTS

FIGURES

TABLES

I. PROJECT OVERVIEW

This project was developed in response to MMS's recognition that offshore oil and gas platforms may serve as a harvestable source of organisms with pharmaceutical or other commercial applications. The project also considered recommendations from the 1998 National Ocean Conference Report (Turning to the Sea: America's Ocean Future, 1999; available at http://www.publicaffairs.noaa.gov/oceansreport/) to (1) increase support for sustainable harvesting and testing of marine compounds by both government agencies and commercial pharmaceutical companies as possible treatments for AIDS, inflammatory or infectious diseases, and cancers; and (2) support research on the environmental effects of extracting marine organisms for biotechnology purposes.

Over 3000 oil and gas platforms in the Gulf of Mexico provide hard substrate for the attachment and growth of large concentrations of fouling organisms in areas otherwise devoid of hard substrate. These platforms, consisting of vertical and horizontal steel members, could form the substrate basis for harvesting organisms with potential for biotechnology applications.

The first objective of this study was, therefore, to identify the organisms that make up the biofouling or epifaunal communities on platforms. The second objective of this study was to preliminarily determine the potential of any of these organisms to be a source of pharmaceuticals or any other commercially important products. Finally, the concentration and distribution of potentially important species on the structures and the variability of that distribution with platform location, density of organisms at various depths on the platform and seasonal variation of occurrence was considered. Knowledge of spatial and temporal variation will be necessary in determining the cost effectiveness of harvesting any potentially important organisms. Alternately, the knowledge of the combination of factors necessary to maximize biomass production of individual species may lead to the design of artificial substrates used exclusively to farm commercially important organisms.

Some of the groups of organisms common to the Gulf of Mexico oil and gas platforms that show potential for biotechnology applications were included in this study. These include bacteria, particularly members of the class Actinobacteria, that have yielded numerous bioactive compounds valued as a source of pharmaceuticals and enzymes; marine algae that provide a range of natural products from agar to pharmaceuticals; benthic foraminiferans, especially agglutinated wall species; byssate molluscs that have potential in the production of bioadhesives for many uses from transdermal drug delivery systems to surgical adhesives; and bryozoans, particularly *Bugula neritina,* a source of bryostatin in some areas, a drug used in cancer treatment.

For this project, six teams of scientists were assembled from the Coastal Marine Institute at Louisiana State University in collaboration with University of Louisiana in Lafayette to address research objectives relative to the chosen taxa. The Project Director was Dr. Lawrence Rouse, and the co-principal investigators were Drs. Fred Rainey (bacteria), Russell Chapman (algae), Barun San Gupta (benthic foraminiferans), Michael Hellberg (bryozoans), Laurie Anderson (molluscs) and David Foltz (genetic analyses of molluscs). Reports of these investigators (and co-investigators) resulting from the relevant studies of their respective taxonomic groups are presented in full with the exception of the platform locations and field collection methods of the samples shared among all investigators and described below.

II. GULF OF MEXICO PLATFORM STUDY SITES AND FIELD COLLECTION METHODS

Two collecting trips were made from Port Fourchon, Louisiana, on M/V *Spree*. On the first cruise, June 9, 2001, Tropical Storm Allison prevented sampling, except at one of the five chosen platforms, ST 23. On the second cruise, May 4-5, 2002, one platform could not be sampled due to dangerous diving conditions. The latitude/longitude locations of the platforms sampled are given in **Table II-1** and their relative locations are shown in **Figure II-1**.

Table II-1

Platform Locations and Water Depths

Platform	Location	Depth (m)	Collection Date
ST 23 (Chevron South Timbalier Block 23)	29º 01.40' N, 90º 10.17' W	15.8	June 9, 2001
ST 67H (ExxonMobil South Timbalier Block 67H)	28º 47.935' N, 90º 24.889' W	19.2	May 5, 2002
GI 42C (Vastar Grand Isle Block 42C)	28º 59.947' N, 89º 56.264' W	30.5	May 4, 2002
GI 82A (El Paso Grand Isle Block 82A)	28º 43.301' N, 89º 57.916' W	51.8	May 5, 2002
GI 95A (Vastar Grand Isle Block 95A)	28º 30.962' N, 90º 07.366' W	61	May 5, 2002

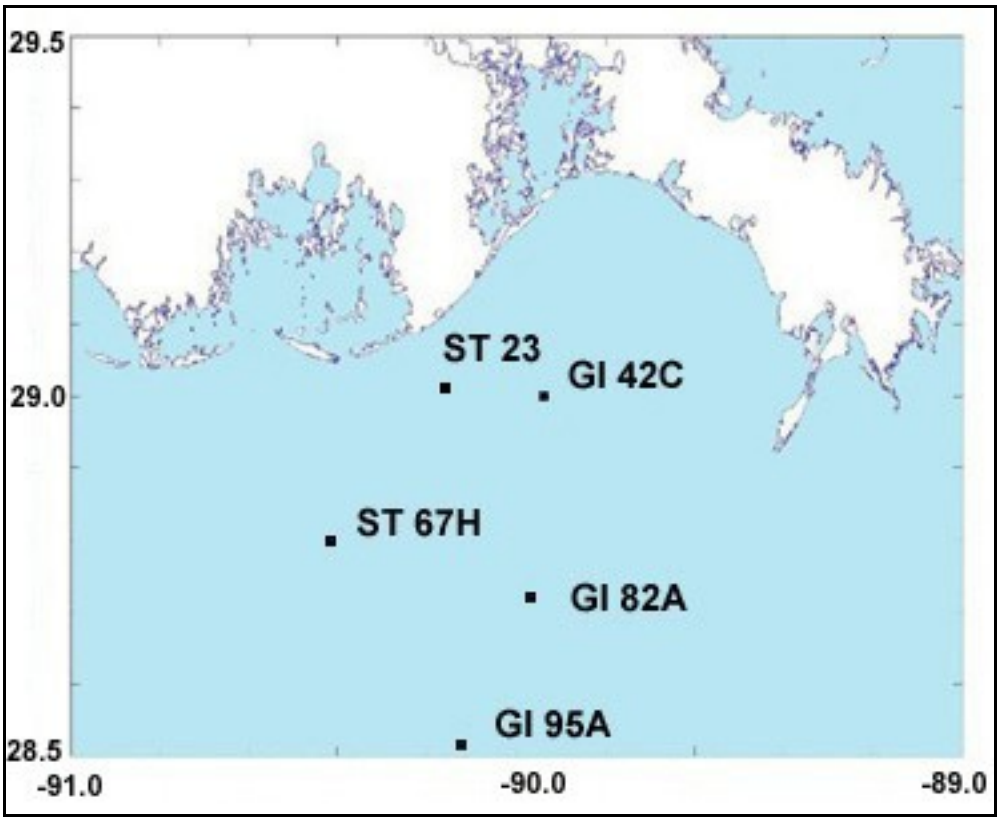


Figure II-1. Platform locations.

Scrapings from an easterly (E) and westerly (W) leg were taken from each platform, using two teams of three divers. Ideally, three levels were to be sampled: near surface (0 m), 10 m, and 20 m. Three (25 cm x 25 cm) areas (A, B, and C) were to be scraped at each sampling level on each leg. This protocol could not be strictly followed due to rough seas. No surface samples were collected at ST 23, and only one surface sample was collected at GI 42C. One surface sample per leg was sampled at the other three platforms. Only five samples were taken at the 10 m level at ST 23 and GI 42C because of diving gear problems. One replicate from 10 m on the west leg of ST 42C was collected but lost before reaching the lab. A summary of the samples collected from each platform leg is given in **Table II-2**. At sites where bottom water depth was less than 20 m, samples labeled "20 m" were actually taken 1 m above the bottom.

Table II-2

Sample Replicates Collected

Platform	Leg	0 m	10 m	20 m
ST 23	E	0	3	3
	W	0	2	3
ST 67H	E	0	3	3
	W	1	3	3
ST 42C	E	1	3*	3
	W	0	2	3
GI 83A	E	1	3	3
	W	1	3	3
GI 95A	E	1	3	3
	W	1	3	3

* Sample Lost in Transit.

At each sampling site, divers outlined a 25 cm X 25 cm area, using a metal template, and the labeled quadrat was photographed. The diver then suctioned loose surface material off this area with a 60 cc syringe. All incrusting organisms were then chiseled off into a large, prelabeled, resealable, plastic bag. At two of the shallower platforms (ST 23 and ST 67), divers collected bottom samples within 1 m of the platform leg, using a 1-l Histoplex jar to scoop up sediment. At these stations, bottom sediment samples were also collected away from the platform, deploying a petite ponar grab from the boat.

Once samples were brought aboard, bacteria subsamples were immediately taken from the scrapings. Each bag of scrapings was then emptied into a plastic tub, an index card with the sample name was added, and any specimens of algae or the bryozoan, *Bugula,* were removed. The remaining contents of the tub were returned to the plastic bag, using seawater to rinse down the sides. The sample was double-bagged, if necessary, and frozen. Syringe samples were placed in an ice water bath. Bottom sediment samples were kept in the 1-liter collection jars and preserved in ethanol (unstained).

These samples were used for analyses of the bacteria, algae, foraminiferans, bryozoans and molluscs in the studies that follow. Additional sampling locations from off the east coast from Florida to Massachusetts for comparative genetic analysis of the bryozoan *Bugula* are addressed in **Section VI**.

III. BACTERIA

Survey of the Numbers and Diversity of Bacteria Associated with the Biomatrix of Oil and Gas Platforms on the Louisiana Continental Shelf

Fred A. Rainey
Department of Biological Sciences
Louisiana State University

INTRODUCTION

Here we report the data obtained on the numbers and diversity of members of the domain Bacteria associated with the biomatrix on oil and gas platforms off the Louisiana coast. This study was part of a multi-taxa investigation to look at the biodiversity associated with gas and oil platforms with the overall aim of using these organisms for biotechnological applications. The specific aims of the project were to (1) determine the numbers and diversity of the bacteria associated with the biomatrix on the oil and gas platforms, (2) determine the location of these organisms with respect to depth above or below the water line, and (3) determine if any of the bacteria associated with the biomatrix have potential for biotechnological applications. There is no previous work described in the literature regarding the bacterial populations associated with the encrusted biomatrix on oil and gas platforms.

METHODS

Sites Sampled

In 2001 the biomatrix on a single platform ST 23 was sampled. The following year in May 2002 five platforms were sampled as described in **Section II**. Samples taken from the biomatrix encrusting the platform legs were very heterogeneous both in structure and macro-organism composition.

Total Counts and Isolation

Ten grams of biomatrix was added to 90 ml of sterile artificial seawater (Instant Ocean) at 4 °C and homogenized using a stainless steel blender for 5 mins. The resulting slurry was serially diluted in sterile artificial seawater before plating on solid media. Two approaches were taken for the analysis of the bacterial populations. One was the approach of determination of the total heterotrophic bacteria that could be sultured from the biomatrix samples and the other was using specific media to target the isolation of specific groups of organisms known to have biotechnological potential. Total counts were determined on Marine Agar (Difco) and one-tenth strength Marine Agar (prepared in full strength artifical seawater). For specific isolations Starch Casein Agar (10g soluble starch, 1g casein, 0.5g K2HPO4, 20g Agar, 1L Instant Ocean) and YME Agar (4g yeast extract, 10g malt extract, 4g dextrose, 20g Agar, 1L Instant Ocean) were used. To these isolation media cyclohexamide (100ug/ml) and rifampicin (5ug/ml) were added. These filter-sterilized antibiotics were added after the agar was autoclaved.

Selection of Isolates

Since numerous isolates have been recovered from isolation studies of marine environment samples a selection strategy was used to attempt to isolate and establish a collection of diverse organisms for further study. Organisms for molecular identification and inclusion in the culture collection were selected on the basis of colony morphological characteristics including colony size, colony color and texture of the colony material.

Identification Using ARDRA and 16S rRNA Gene Sequencing

Genomic DNA was isolated from the strains and the 16S rRNA gene amplified using PCR. The ARDRA technique was used to group the isolates and reduce duplication prior to sequencing. The amplification and sequencing was done as described by Rainey et al. (1996). The 16S rRNA gene sequences were compared with all available sequences in the GenBank database using the BLAST tool (Altschul, et al., 1990).

Establishment of a Culture Collection

A culture collection was established in which each of the isolates selected for identification was purified and preserved in liquid media containing 15% (v/v) glycerol at -80 °C. The strains have been preserved in triplicate.

RESULTS

Bacterial Counts

Total colony forming units (cfu) per gram of biomatrix were determined for each of the five platforms studied. Counts were determined for samples taken at 0, 10 and 20 meters and at one meter above the surface for sites in less than 20m of water (ST23 and ST67H). Samples were dilution plated on Marine Agar or 1/10 strength Marine Agar. The 1/10 strength Marine Agar contains a lower amount of carbon and energy sources (sugars and yeast extract) and was used based on previous observations in attempts to recover novel organisms from low nutrient environments. The total colony forming units per gram of biomatrix for all five platforms sampled ranged from 5.8×10^4 to 9.0×10^6. The lowest numbers of cfus were found for platform GI82A-W at a depth of 20m (plated on Marine Agar) and the highest counts were found for platform GI42CW at a depth of 10m (plated on 1/10 strength Marine Agar). **Figure III-1** shows a graphical representation of the cfu/g of biomatrix for the platforms GI-82Aand GI-42C. The E and W designations indicate the east and west supports of the platform. From the comparison of these two data sets we can see there are significant differences in the numbers of colony forming units recovered from the different platforms, the different locations (east and west support structures) and the depth sampled. In the case of samples from the west support structure of platform GI-82A it can be seen on both MA and 1/10 MA the cfu/g of biomatrix are significantly lower than that found on the east support structures of the same platform at a depth of 20m. Interestingly at 10m the reverse is the case with the higher numbers of cfu/g of biomatrix being recovered from the west support structures.

These differences between depths and east/west location are a common trend in the data sets obtained. The numbers of cfu/g of biomatrix recovered will be directly related to the macro composition of the samples from which the bacteria are isolated. Samples containing higher

numbers of macro scale organisms will contain higher numbers of culturable bacteria when compared to samples containing low numbers of living macro scale organisms.

Diversity of Organisms Recovered

The biomatrix of the platforms studied harbor an extensive diversity of bacterial species. This is not unexpected considering the large diversity of bacteria present in the majority of marine environments. The biomatrix does provide a location for the accumulation of diversity that in the case of the open ocean would be much more dilute and require extensive concentration to recover a similar level of diversity. The use of 16S rRNA gene sequencing allows us to realize this diversity and put it into a taxonomic context. When looking at the diversity in terms of similarity to other species or strains it should also be considered that strains of the same species can produce very different secondary metabolites and bioactive products.

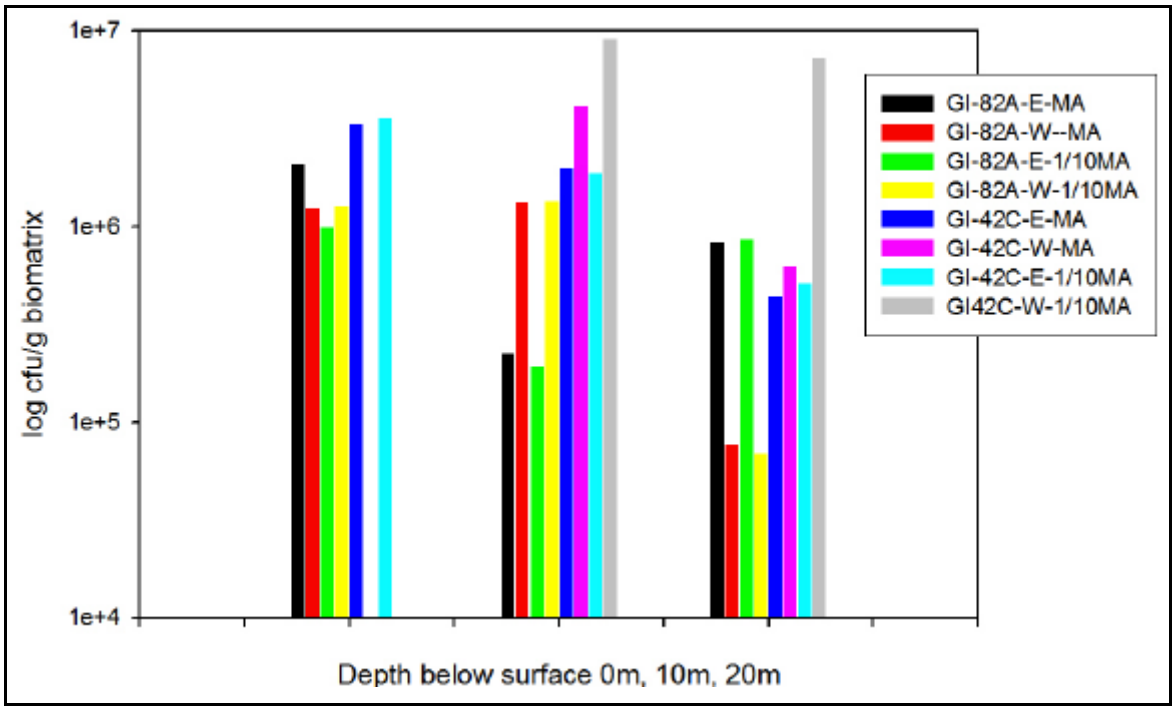


Figure III-1. Variation in cfu/g biomatrix with depth and culture media for Platforms GI 82A and GI 42C.

From the serial dilution plate counts on Marine Agar and 1/10 strength Marine Agar bacteria from three phyla of the domain Bacteria are recovered. The phyla represented by these isolates are the Firmicutes, Proteobacteria and Actinobacteria. The isolates for further study were selected based on the diversity of their colony morphologies when grown on agar plates. The majority of the isolates, 69% of them fall within the phylum Proteobacteria and represent species of genera which are typically found in the marine environment eg. *Vibrio, Photobacterium, Pseudoalteromonas, Microbulbifer, Shewanella and Ruegeria*. Some 29% of the isolates from serial dilution plating are representatives of species of genera in the Firmicutes phyla. These isolates are mostly *Bacillus* species with a small number of *Plannococcus* species. The remaining 2% of the isolates are actinobacteria of the genera *Streptomyces* and *Gordonia*. **Figure III-2**

provides an example of the diverse types of colony morphologies observed in this isolation study.

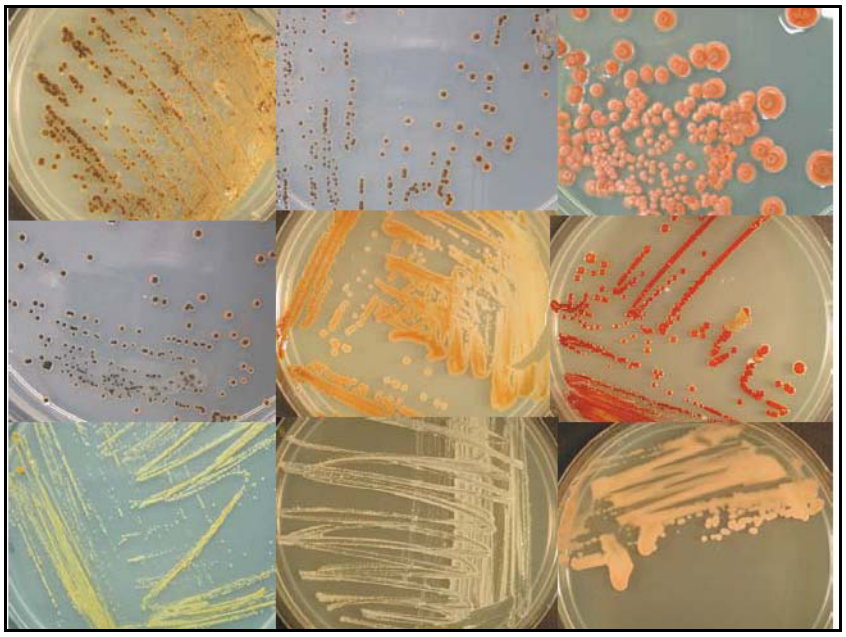


Figure III-2. Diverse colony morphologies observed in this isolation study.

Using the more selective approach of additional culture media (as described in methods section) that contained the antibiotics cyclohexamide and rifampicin the Gram negative members of the Proteobacteria were eliminated and large numbers of Gram positives especially actinobacteria that were only represented at low levels on Marine Agar cultivation could be enriched. It is these actinobacteria that have traditionally been a great source of secondary metabolites and bioactive compounds. Using this approach we cultivated a large number (156) of actinobacteria including members of the genera *Streptomyces*, *Micromonospora*, and *Agromyces*. Species of the genera *Streptomyces* and *Micromonospora* are well known for their production of bioactive molecules including antibiotics. Based on their 16S rRNA gene sequence data these isolates represent new species of these genera and therefore have the potential to produce novel compounds.

Products of This Study

Novel Isolates

Although the isolates for the most part represent species of already described genera the majority of them have 16S rRNA gene sequence similarities of less than 98% to other described species. 16S rRNA gene sequence similarity values below 98% point to the novelty of these isolates at the species level and in some cases at the genus level. The large number of actinobacteria that can be recovered from the biomatrix on these platforms is encouraging both in the fact that these organisms are part of the renewable biomatrix but in addition these actinobacterial components contain novel and as yet undescribed species. The large number of *Bacillus* species could be by common view written off as insignificant with respect to biotechnological applications. However, in collaboration with a colleague at University of

Louisiana Monroe (ULM) we have found similar *Bacillus* strains from sea sponges that act as biocatalysts producing compounds that have anti-tumor properties (Elsayed et al., 2007). These novel isolates represent a resource that is currently being studied both at LSU and at ULM for their biotechnological applications. In 2003 as a result of the data obtained in this project the Louisiana BOR (through the Governor's Biotechnology Initiative) funded a permanent faculty line and a technical line in the area of natural products science at LSU.

Novel Strain Collection

A collection of some 400 strains (purified and preserved) has been established at LSU. The number of strains from each of the five platforms sampled is shown in **Table III-1**. Some of these strains have been provided to researchers at LSU and ULM for further study. A database of the strains, source and their growth conditions has been established.

Table III-1

Number of Strains Collected on Each Platform Sampled

Platform	Strains Included in the Collection
GI 82A	81
GI 95A	31
GI 42C	78
ST 23	101
ST 67H	109

CONCLUSIONS

The study demonstrated that the biomatrix associated with oil and gas platforms contains large numbers of bacterial species many of which are novel and belong to taxonomic groups known to be of biotechnological importance. There are a number of factors that would seem to influence the bacterial load and diversity of the biomatrix recovered from a given location. The composition and density of the macroorganisms would seem to influence the numbers and diversity of the bacterial species recovered by conventional isolation techniques. The isolation approach used also influences the types and diversity of the strains recovered. The use of general culture media such as Marine Agar provides both proteobacterial and Gram positive bacteria with the majority being Proteobacteria which are known to be dominant in marine environments. Using a selective approach can result in the isolation of more Gram positives and actinobacterial species which in general are considered to have greater potential for biotechnological applications. This study clearly shows that the biomatrix on the structures of oil and gas platforms is the habitat of novel bacterial species that represent a biotechnological resource waiting to be exploited. Isolation and natural product screening still has its place in biotechnology but the application of whole genome studies to such environments is the way forward. The construction of total genome libraries from the biomatrix of oil and gas platforms and their expression will no doubt provide novel bioactivities from both the micro and macro components of these complex systems. Here we have established that the biomatrix encrusted on oil and gas platforms represents a potential starting material for such studies.

REFERENCES

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.

El Sayed K.A., S. Laphookhieo, H.N. Baraka, M. Yousaf, A. Hebert, D. Bagaley, F.A. Rainey, A. Muralidharan, S. Thomas, and G.V. Shah. 2008. Biocatalytic and semisynthetic optimization of the anti-invasive tobacco (1S,2E,4R,6R,7E,11E)-2,7,11-cembratriene-4,6-diol. Bioorganic & Medicinal chemistry 16(6):2886-2893.

Rainey, F. A., N. Ward-Rainey, R. M. Kroppenstedt, and E. Stackebrandt. 1996. The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam. nov. International Journal of Systematic Bacteriology 46:1088-1092.

IV. MACROALGAE

Biodiversity and Potential Biotechnological Uses of Marine Macroalgae from the Offshore Oil and Gas Platforms in the Gulf of Mexico

Juan M. López-Bautista[1] and Suzanne Fredericq
University of Louisiana at Lafayette

Russell L. Chapman[2] and Debra A. Waters
Louisiana State University

INTRODUCTION

The more than 3000 standing oil and gas platforms in the northwestern Gulf of Mexico (GOM) provide habitat for a significant community of marine organisms. As part of a larger study to determine the potential for organisms inhabiting oil rigs to provide a harvestable source of organisms with biotechnology potential, this study was designed to determine species diversity, relative abundance, and vertical, horizontal, and seasonal distribution of macroalgae.

METHODS

The May 2002 cruise visited four platforms where algae were collected GI 42C, GI 82A, GI 95A, ST 23, and ST 67H (**Figure II-1**).These sites were selected to follow a horizontal gradient that allowed comparison of near-shore to outer-shelf assemblages.

At each site, divers sampled the east-west legs of the platform as described in **Section I.** After sample bags were carried on board, the algal component was selected with forceps or spatulas and transferred to labeled bags. The thalli were sorted as follows:

- 48 sample bags fixed in 90% ethanol for algal identification, curation on semi-permanent microscope slides, and DNA extraction and molecular study;
- 14 sample bags of algae were kept alive in a cool chamber in seawater for further culture studies in the laboratory at University of Louisiana at Lafayette (ULL).

Cultures were maintained at 18^{o}C with a light/dark regime of 16/8 hrs in a Percival Scientific benchtop plant growth chamber Model E-30B in seawater enriched with Alga/Gro (Carolina Biological Supply Co.). A collection of isolates in a culture chamber was kept in seawater medium for further detailed studies. DNA studies for ongoing molecular systematic analysis were done on some samples using the following methods:

For the DNA extraction, a small portion of the algal sample was ground in liquid nitrogen with a mortar and pestle. DNeasy Plant Mini Kit from QIAGEN was used following the manufacturer's protocol. For gene amplification, 2:l of the resulting extraction was used as template for a 50:l PCR consisting of 10:l 5M betaine, 6:l 10x PCR buffer (Perkin Elmer Corp.),

[1] Currently at the University of Alabama, Tuscaloosa, Alabama.
[2] Currently at Scripps Institutions of Oceanography, University of San Diego, California.

6:l 25 mM MgCl2 solution, 8:l of 500 mM dNTP stock, 2:l each of the appropriate primers at 10 mM, and 0.3-0.5:l Amplitaq® DNA Polymerase. Amplification conditions for rbcL consisted of 4 minutes at 96°C for denaturation, followed by 30 cycles of 60 seconds at 94°C, 60 seconds at either 45°C or 42°C, and 90 seconds at 72°C, with a final 10 minute extension cycle at 72°C, and soak cycle at 4°C. The amplification reactions were performed on a PE GeneAmp PCR system 9700 or 2400. For automated gene sequencing, amplification products were cleaned of excess primer, enzyme, and dNTPs by PEG precipitation (Hillis et al., 1996).

The sequences were determined over both strands using an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, CA, USA). Reaction mixtures comprised 4 :l Terminator Ready reaction mix with 4 :l 2.5X buffer or 8:l Terminator Ready reaction mix, 1-2 :l template, 3.2 pmol primer, and deionized water q.s. up to a total volume of 20 :l. The cycle sequencing reactions were performed on a PE GeneAmp PCR system 9700 or 2400 for 25 cycles (96°C for 10 seconds, rapid thermal ramp to 50°C, 50°C for 5 sec., rapid thermal ramp to 60°C, 60°C for 4 min, rapid thermal ramp to 4°C). Resulting products were then purified using Centri-Sep spin columns (Princeton Separations P/N CS-901) following the manufacturer's instructions.

RESULTS

A total of 24 taxa have been identified from the samples. **Table IV-1** is a list of algal taxa found. The number of taxa by division/class were as follows:

- Chlorophyta: 39
- Rhodophyta: 42
- Phaeophyceae: 19
- Cyanophyta: 3

The macroalgae collected from the platforms are known to occur in the northwestern GOM with the exception of one red algal species, *Antithamnionella breviramosa.* It is reported here for the first time from the GOM. It was previously known from Pacific Mexico, eastern Australia, the Solomon Islands, North Carolina, and Brazil. Six other species, although known to occur in the Gulf of Mexico, were reported for the first time from the oil and gas platforms in Louisiana. They included *Ulva fasciata, Chaetomorpha aerea, Gelidium pusillus, Jania capillacea, Callithamniella tingitana, Centrocerus clavulatum*. All but one belong to genera with biotechnological potential.

Table IV-1

Algal Taxa Collected

Chlorophyta	**Bryopsis pennata* Lamouroux **Chaetomorpha aerea* (Dillwyn) Kützing **Cladophora* sp. **Enteromorpha* spp. *Entocladia viridis* Reinke **Ulva fasciata* Delile *Ulvella lens* P. Crouan & H. Crouan
Rhodophyta	*Acrochaetium hypneae* Børgesen *Acrochaetium microscopicum* (Nageli ex Kützing) Nägeli [+]*Antithamnionella breviramosa* (Dawson) Wollaston in Womersley & Bailey+ *Callithamniella tingitana* (Schousboe ex Bornet) Feldman-Mazoyer **Centroceras clavulatum* (C. Agardh in Kunth) Montagne in Durieu de Maisonneuve **Ceramium flaccidum* (Kützing) Ardisson *Erythrotrichia carnea* (Dillwyn) J. Agardh **Gelidium pusillum* (Stachouse) Le Jolis *Herposiphonia secunda* (C. Agardh) Ambronn f. *tenella* (C. Agardh) Wynne **Jania capillacea* Harvey *Kylinia crassipes* (Børgesen) Kylin *Sahlingia subintegra* (Rosenvinge) Kornmann
Phaeophyceae	*Kuetzingiella elaschistaeformis* (Heydrich) Balakkrishnan & Kinkar *Sphacelaria rigidula* Kützing
Cyanobacteria	*Gloeocapsa* sp. *Oscillatoria acuminata* Gomont *Spirulina* sp.

[+]First report from the Gulf of Mexico.
*Taxa have been reported as being of biotechnological potential.

Approximately half of the macroalgae collected from the platforms are known to have biotechnological potential. Some of their properties that are or may be of potential use are given below.

- **Agar Producing**: *Gelidium* spp. (Cooper and Johnstone, 1944)
- **Antibacterial**: *Cladophora* spp., *Gelidium* spp. (Trono, 1999), *Centroceras clavulatum* (Caccamese et al., 1981)
- **Antifungal**: *Bryopsis* spp. (Tringali, 1997), *Cladophora* spp. (Trono, 1999; Ballesteros et al., 1992), *Centroceras clavulatum*, (Caccamese et al., 1981), *Gelidium pusillum*, *Jania* spp. (Ballesteros et al., 1992)
- **Antiherpetic**: *Chaetomorpha* spp., *Cladophora* spp., *Eneromorpha* spp., *Centroceras clavulatum* (Hayashi et al., 1996), *Ulva fasciata* (Santos et al., 1999)
- **Anti-inflammatory**: *Gelidium* spp. (Baker, 1984)

- **Anti-influenza**: *Ceramium* spp., *Gelidium* spp. (Serkedjieva et al., 2000)
- **Antitumoral**: *Bryopsis* spp. (Tringali, 1997), *Enteromorpha* spp. (Hagashi-Okai et al., 1999)
- **Antiviral**: *Bryopsis* spp, *Ulva fasciata* (Tringali, 1997), *Cladophora* spp., *Gelidium* spp. (Trono, 1999), *Enteromorpha* spp. (Kim et al., 1997), *Centroceras clavulatum* (Caccamese et al., 1981)
- **Diuretic**: *Enteromorpha* spp. (Naqvi et al., 1980). Additionally, the agar produced from red algae is widely known to have antirheumatic properties and alginates from brown algae (Phaeophyceae) have been studied for spinal chord, and nerve regeneration (Novikov et al.; 2002), bone repair (Felicio-Fernandex and Laranjeira, 2000) and angiogenesis (Zemani et al., 2005). *Spirulina* (Cyanophyta) is used as a food additive (Miranda et al, 1998).

Figure IV-1 shows the vertical distribution of the algae commonly collected at the platforms.

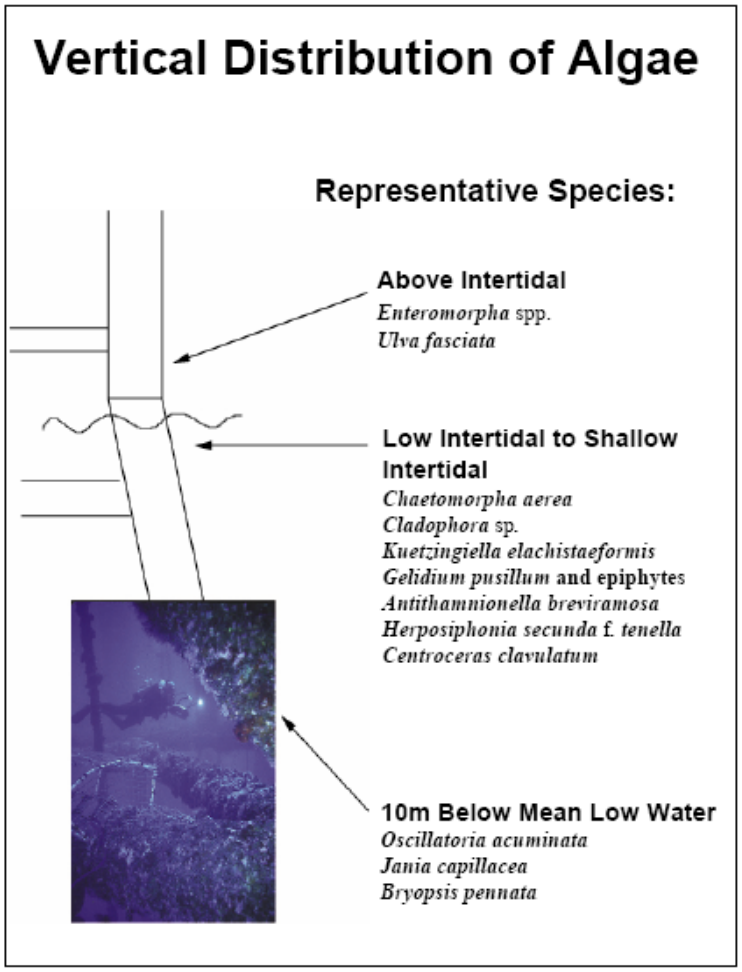


Figure IV-1. Vertical distribution of algae.

A zonation of algal communities in the intertidal area was visible at all stations and consisted of barnacles and small blades of green algae, mainly *Ulva fasciata* and several species of

Enteromorpha. From low intertidal to shallow intertidal, common algae included *Chaetomorpha aerea, Cladophora sp., Kuetzingiella elachistaeformis, Gelidium pusillum and epiphytes, Antithamnionella breviramosa, Herposiphonia secunda f. tenella and Centroceras clavulatum.* From 10 m below mean low water, the most common species were *Oscillatoria acuminata, Jania capillacea and Bryopsis pennta.*

In addition to the vertical distribution of the algae on the platforms, the biodiversity and abundance of macroalgae was found to increase from nearshore to offshore and decrease from intertidal to depth.

CONCLUSIONS

The total number of taxa collected during this study was 24. The best represented group was Rhodophyta (approximately 50% of the taxa collected). There was a vertical distribution of taxa found on the platforms themselves, and there was also a trend identified for biodiversity and abundance of macroalgae to increase from nearshore to offshore and decrease from intertidal to depth.

In all, seven new taxa were added to the list of macroalgae identified from the platforms and one new report of *Antithamnionella breviramosa* from the Gulf of Mexico. Approximately 50% of the taxa collected are known to have biotechnological potential and approximately 20% of the taxa collected are found only on oil and gas platforms.

The macroalgal taxa that occur on the platforms in the Gulf of Mexico are still not well known. There is a need for further work on this group including comparisons of offshore and nearshore macroalgae and seasonal diversity as well as determining the importance of these macroalgae to herbivores. With further elucidation of the taxa that occur in the Gulf, their potential use in biotechnology can be more definitively assessed.

REFERENCES

Baker, J.T. 1984. Seaweeds in pharmaceutical studies and applications. Hydrobiologia 116/117:29-40.

Ballesteros, E., D. Martin, and M.J. Uriz. 1992. Biological activity of extracts from some Mediterranean macrophytes. Botanica Marina 35:481-485.

Caccamese, S., R. Azzolina, G. Furnari, M. Cormaci, and S. Grasso. 1980. Antimicrobial and antiviral activities of extracts from Mediterranean algae. Botanica Marina 23:285-288.

Cooper, N.C., G.R. Johnstone. 1944. The seasonal production of agar in *Gelidium carthilagineum*, a perennial red alga. American Journal of Botany 31(10):638-640.

Felicio-Fernandez, G., and Laranjeira, M.C.M. 2000. Calcium phoshophate biomaterials from marine algae. Hydrothermal synthesis and characterization. Quinn. Nova 23(4):441-446.

Hayashi, K., J. Hamada, and T. Hayashi. 1996. A screening strategy for selection of anti-HSV - 1 and anti-HIV extracts from algae. Phytotherapy Research 10(3):233-237.

Haqashi-Okai, K, S. Otani, and Y. Okai. 1999. Potent suppressive effect of a Japanese edible seaweed, Enteromorpha prolifera (Sujiaopnori) on initiation and promotion phases of chemically induced mouse skin tumonigenesis. Cancer Letters 140(1):21-25.

Hillis, D.M., B.K. Mable, A. Lason, S.K. Davis, and E.A. Zimmer. 1996. Nucleic Acids IV: sequencing and cloning. pp. 321-381. In Hillis, D.M., C. Moritz, and B.K. Mable, eds. Molecular Systematics. Sinauer Associates, Inc. Sunderland MA. 655 pp.

Kim, J.H., J.B. Hudson, A.M. Huang, K. Bannister, H. Jin, T.J. Choi, G.H.N. Towers, Y.K. Hong, and R.E. DeWreede. 1997. Biological activity of seaweed extracts from British Columbia, Canada and Korea. I. Antiviral activity. Canadian Journal of Botany. 75(10): 1656-1660.

Miranda, M.S., R.J. Cintra, S. B. M. Barros, and J. Mancini-Filho. 1998. Antioxidant activity of the microalga *Spirulina maxima.* Brazilian Journal of Medical and Biological Research. 31(8):1075-1079.

Naqvi, S.W.A., S.Y. Kamat, L. Fernandes, and C.V.G Redsdy. 1980. Screening of some marine plants from the Indian coast for biological activity. Botanica Marina 24:1-55.

Novikov, L.N., L. N. Novikova, A. Mosahebi, M. Weiberg, G. Terenghi, and J. Kellerth. 2002. A novel biodegradable implant for neuronal rescue and regeneration after spinal chord injury. Biomaterials: 23(16):3369-3376.

Serkedjieva, J., M. Konaklieva, S. Dimitrova-Konaklieva, V. Ivanova, K Stefanov, and S. Popov. 2000. Antiinfluenza virus effect of extracts from marine algae and invertebrates. Z. Naturforsch 55c:87- 93.

Santos, M.G.M., M. H. C. Lagrota, M.M.F. Miranda, Y. Yoneshigue-Valentin, and M.D.A. Wigg. 1999. A screening for the antiviral effect of extracts from Brazilian marine algae against a cyclovir resistant herpes simplex virus type 1. Bot Mar. 42:iii-v.

Tringali, C. 1997. Bioactive Metabolites from Marine Algae: Recent Results. Current Organic Chemistry 1:375-395.

Trono, G.C. 1999. Diversity of the seaweed flora of the Philippines and its utilization. Hydrobiologia 398-399:1-6.

Zemani F., D. Benisvy, I. Galy-Faroux, A. Lokajczyk, S. Colliec-Jouault, G. Uzan, A.M. Fischer, and C. Boisson-Vidal. 2005. Low-molecular-weight fucoidan enhances the proangiogenic phenotype of endothelial progenitor cells. Biochemical Pharmacology 70(8):1167-1175.

V. FORAMINIFERA

Evaluation of Oil and Gas Platforms on the Louisiana Continental Shelf for Organisms with Biotechnology Potential

Barun K. Sen Gupta
Department of Geology and Geophysics
Louisiana State University

and

Lorene E. Smith
Museum of Natural Science
Louisiana State University

INTRODUCTION

Benthic foraminiferal communities of the northwestern Gulf of Mexico continental shelf have been the subject of numerous studies, mainly with regard to broad patterns of geographic and water-depth distributions, but also in the context of ecological questions such as the effects of seasonal hypoxia on populations. There is, however, no published account of foraminiferal species living on oil and gas platforms in the Gulf. With this background, the focus of the present investigation was the taxonomic diversity and dominance trends of such species. Particular attention was given to species with agglutinated wall structure, because of their potential as a source of bioadhesives.

METHODS

Laboratory Methods

In the laboratory, samples of scrapings were thawed, washed over a series of sieves (63 μm, 500 μm, 1 mm, and 2 mm), and preserved in Rose-Bengal-stained ethanol. One replicate from each depth and platform was selected for analysis. After a minimum of 48 hours, the two smaller size fractions (63–500 μm and 500 μm –1 mm) were rinsed, oven dried, and examined using a dissecting stereomicroscope. All benthic foraminifers, loose and attached, were removed. If the foraminifers were especially numerous, the fraction was subsampled using a microsplitter. For five selected samples, the larger size fractions were also picked. Selected species were illustrated by digital photomicrography and scanning electron micrography.

RESULTS

Assemblage Density

The degree of platform leg fouling varied from site to site. A rich assemblage of benthic Foraminifera was recognized in the scrapings and the syringe samples; the species included both grazers and attached forms. The assemblage density was highly variable. The estimated density for the fine (63 μm – 1 mm) fraction in 19 samples varied between 2,112 and 107,488/m^2. The estimated density for both fine and coarse fractions in five samples varied between 15,728/m^2 and 143,920/m^2 (**Figure V-1**). The gray bars for 14 samples represent only the fine (63 μm – 1 mm) fractions; for five samples, the estimates for the coarse fraction (>1 mm) are black bars.

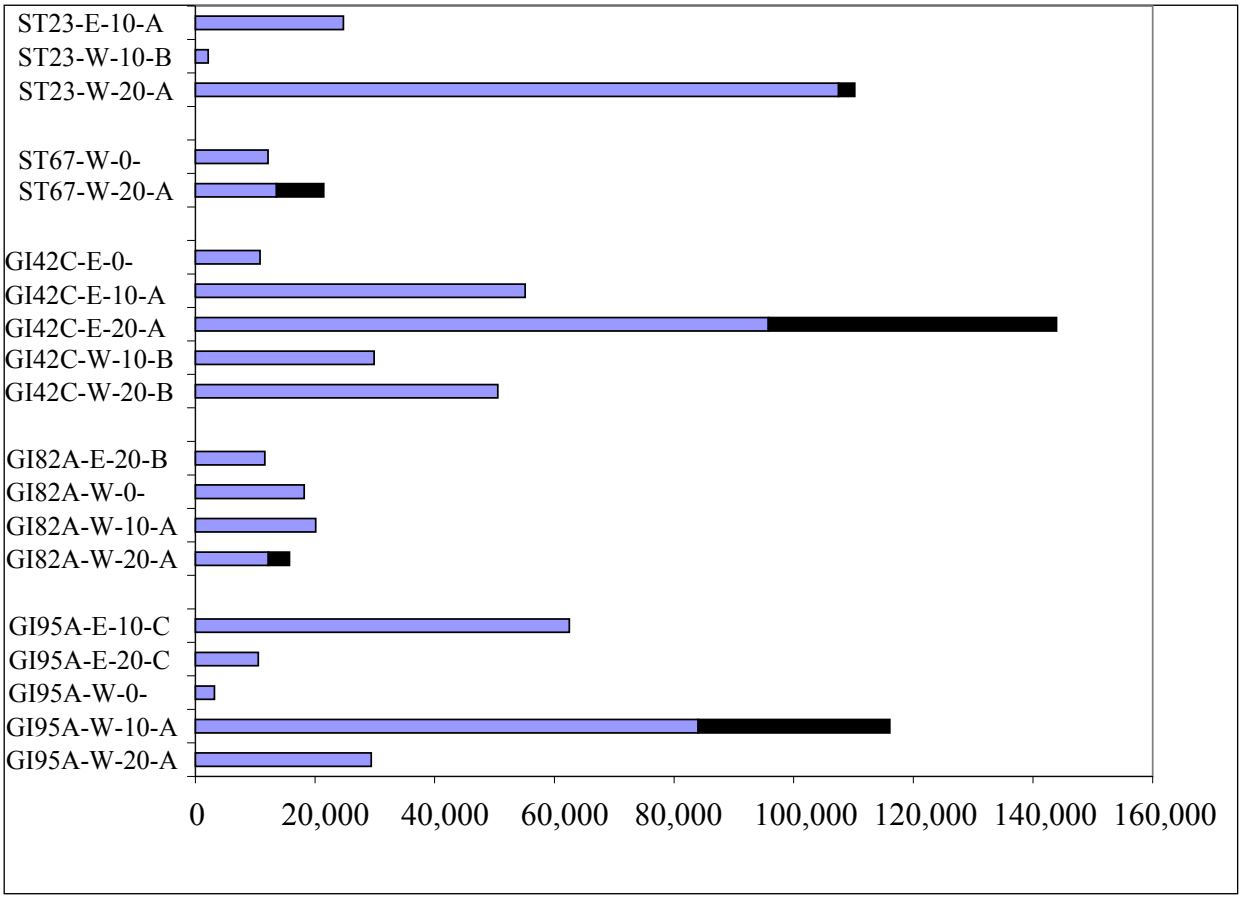


Figure V-1. Estimated foraminiferal density (number/m^2) on platform surface.

The proportion of living (i.e., Rose-Bengal-stained individuals) to total foraminiferal counts in these five samples varied between 12% and 70% (**Table V-1**). Given the small number of samples with living foraminiferal data, generalizations are hard to make, but we note that the relative abundance of living Foraminifera was lowest at the base of the shallowest platforms. These populations were possibly affected by hypoxia that is known to prevail in the area in spring and summer (Rabalais et al., 2002).

Table V-1

Foraminiferal Density Data from Five Samples

Sample	Fine and Coarse Fractions			
	No. of Forams Picked	Forams / m^2	% Living	Living Forams / m^2
ST 23 -W-20-A	3,321	110,208	11.9	13,120
ST 67-W-20-A	924	21,440	29.3	6,288
GI 42C-E-20-A	6,023	143,920	45.8	65,936
	983	15,728	70.3	11,056
G I95A-W-10-A	4,636	116,048	45.2	52,416

Foraminiferal Diversity and Dominance

Other than allogromiids (species with organic-walled shells), all major groups (agglutinated, porcelaneous, and calcareous shells) were represented in the samples. An abundance of juveniles and extraordinary morphological variations in attached species make species-level taxonomy particularly difficult, but we recorded over 20 species in most samples; the highest number was 51 in sample GI95A-W10A. Free-moving species (grazers) were dominated by *Ammonia parkinsoniana*, *Astrononion gallowayi*, *Bolivina spp., and Eubuliminella morgani*. Attached species were dominated by *Cibicides* spp. (especially *C. lobatulus*), *Cornuspira involvens*, *Patellina corrugata*, *Planorbulina* spp., and *Trochammina* sp.

Attached Species

The most striking difference between the foraminiferal assemblage of the platforms and that of seafloor sediment from the area is in the abundance and diversity of attached species. These species are listed below:

(1) Agglutinated group: *Ammobaculites* sp. cf. *A. agglutinans* (**Figure V-2a**), *Glaphyrammina americana* (**Figure V-2b**), *Placopsilina* sp (**Figure V-2c**), *Sagenina divaricans* (**Figure V-2d, e**), *Textularia* sp. (**Figure V-2f**), *Trochammina* sp. (**Figure V-2g**).

(2) Calcareous group: *Acervulina* sp. (**Figure V-3a**), *Calcituba polymorpha* (of the type described by Arnold (1967); **Figure V-3b-e**), *Cibicides lobatulus* (**Figure V-3f, g**), *Cornuspira involvens* (**Figure V-4a**), *Dyocibicides* sp., *Hanzawaia concentrica* (**Figure V-4b**), *Melonis* sp. (**Figure V-4d**), *Neoconorbina* sp. (**Figure V-4c**), *Nodobacularia bonairensis* (**Figure V-4e-g**), *Nubeculinita* sp. cf. *N. inhaerens* (**Figure V-5a**), *Parrina bradyi* (**Figure V-5b, c**), *Patellina corrugata* (**Figure V-5d**), *Planorbulina mediterranensis* (**Figure V-5e, f**), *Rectocibicides* sp. cf. *R. miocenicus* (**Figure V-6a**), *Rosalina globularis* (**Figure V-6b,c**), *Rosalina* sp. (**Figure V-6e**), *Spirillina vivipara* (**Figure V-7d**), *Webbinela rugosa*, unidentified miliolids.

Most attached Foraminifera adhere to living or dead barnacles (e.g., **Figures V-2c,g, V-3d,e, V-4c, f**, **V-5a, V-6b, c,d**) and bivalves (e.g., **Figure V-2b, V-4a,e, V-6a**) that cover the

platforms. Other foulers that provide attachment surfaces to the Foraminifera include sponges, tunicates, erect bryozoans (**Figure V-3g**), encrusting bryozoans (**Figures V-3b,c**), hydroids (**Figures V-3a,f**), algae (**Figure V-5c**), calcareous and chaetopterid polychaete tubes (**Figures V-5e, V-6e**, respectively), unidentified shell fragments (**Figure V-2a**,**d-f**, **V-4b,g, V-5d,f**), and motile organisms such as crabs.

Within the succession of fouling, Foraminifera were found both on top and beneath invertebrate settlers. Firmly cemented foraminifers (e.g. *Sagenina divaricans*, *Placopsilina* sp., Nubeculariidae, some tube-like foraminifers) were typically present on more exposed, harder substrates. *Planorbulina* spp. were usually found firmly attached to hard substrates such as barnacle and mollusk shells, coralline algae, and calcareous polychaete tubes, but they were occasionally found on sponges. At one station, *Melonis* sp. was attached to a bivalve (probably arcid) byssus (**Figure V-4d**). At sites where barnacles were covered with sponges, tunicates, and octocorals, loosely attached *Spirillina*, *Cornuspira*, and miliolids were more common. *Rosalina globularis* was loosely attached to both calcareous and non-calcareous substrates. This species was also found in pits on shell surfaces. For some foraminiferal species (e.g., *Planorbulina mediterranensis, Acervulina* sp., *Cibicides* spp., *Rosalina globularis*), the shell morphology is modified by the shape of the attachment surface; misshapen shells and aberrant chamber arrangements are common.

Discussion

The most conspicuous aspect of the foraminiferal distribution is the abundance of attached taxa; some sessile species that are rare or absent in soft substrates live in relative profusion on the platforms. In addition, the foraminiferal community includes many vagrant foraminiferal species that have been recruited from soft-bottom natural substrates. Many sessile species of Foraminifera secrete an organic film for attachment (e.g., Poag, 1982), and are easily detached from the substrate by mechanical forces. Our sample coverage may be inadequate for a proper census of these species. The distribution of errant polychaetes (Gallaway and Lewbel, 1982) suggests that depressions and crevices on the surface of the artificial reefs (i.e., on or between the surfaces of live or dead, shelled epifauna) would be particularly suitable microhabitats for Foraminifera, providing both food and shelter. Judging by the high diversity of motile species on natural reefs (e.g., Rose and Lidz, 1977; Hallock, 1999; Sen Gupta, 1999), it is likely that further work would reveal the presence of a large suite of motile species on GOM platforms, feeding on bacteria, microscopic algae (including diatoms), and particulate organic debris (Goldstein, 1999).

We postulate that foraminiferal colonization of platform legs starts with (1) migration of individuals from the surrounding seafloor, (2) settlement of benthic zygotes following the union of free-swimming gametes (or settlement of benthic gamonts produced by meiosis), (3) transport by motile invertebrates (e.g., mollusks, arthropods), or (4) accidental settlement of living individuals after the resuspension of physically disturbed seafloor sediment (Brunner and Biscaye, 1997). A relevant question is the distance of migration between the natural habitat (provenance) of a species and the platform (artificial reef). Published data from the Flower Garden Banks, the nearest living coral reef (Poag and Tresslar, 1981), show that many species present there are also present on the platforms, but these are all widespread species of the Gulf of

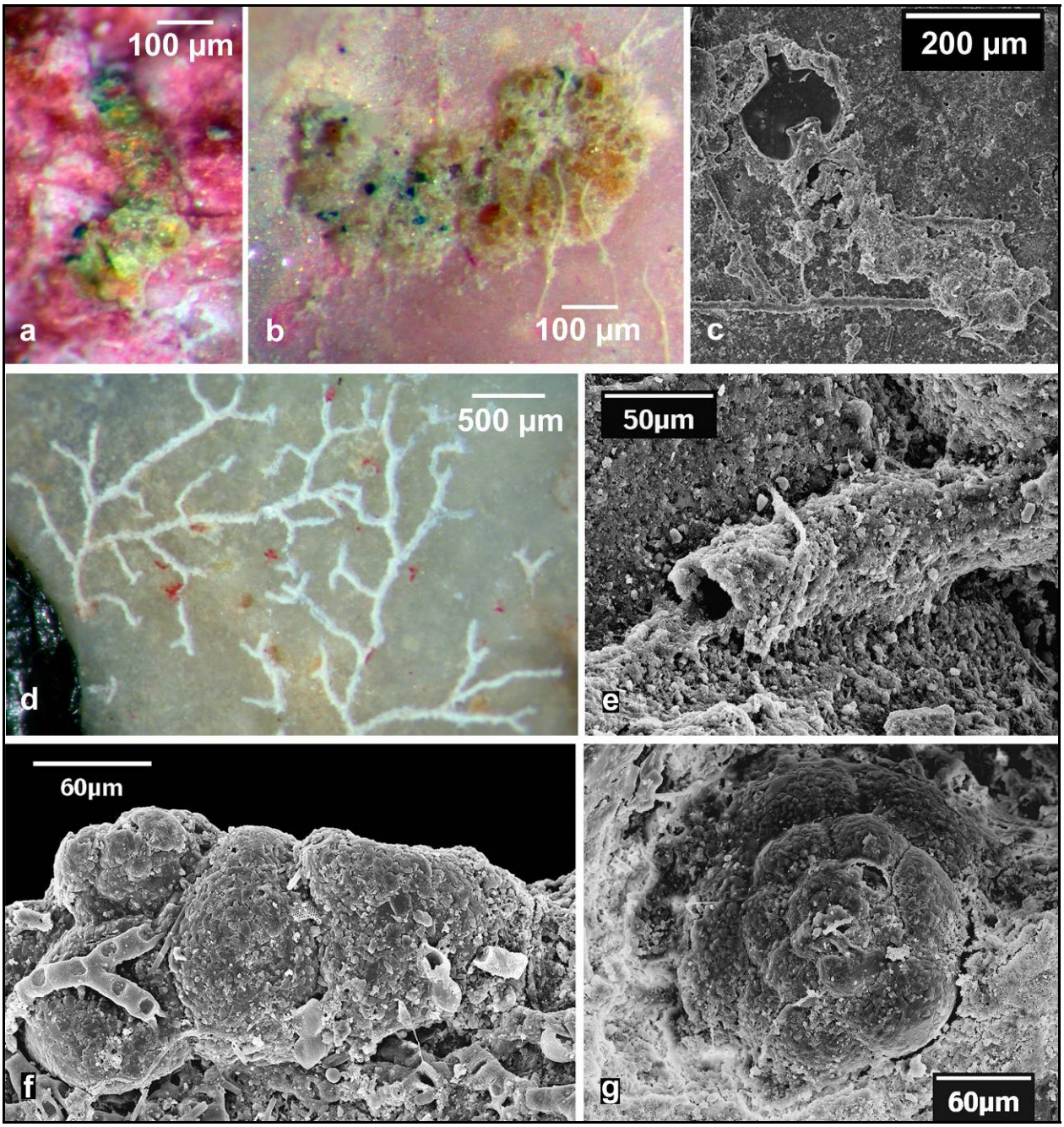


Figure V-2. **Attached agglutinated species; a, b, d, digital photomicrographs, others scanning electron micrographs. a, *Ammobaculites* sp. cf. *A. agglutinans* (GI 95A-W10A); b, *Glaphyrammina americana* (ST 67H-W20A); c, *Placopsilina* sp. (GI 95A-W10A); d, e, *Sagenina divaricans* (d: GI 95A-W10A, e: GI 42C-E20A); f, *Textularia* sp. (ST 67H-E10B); g, *Trochammina* sp.(GI 95A-W10A).**

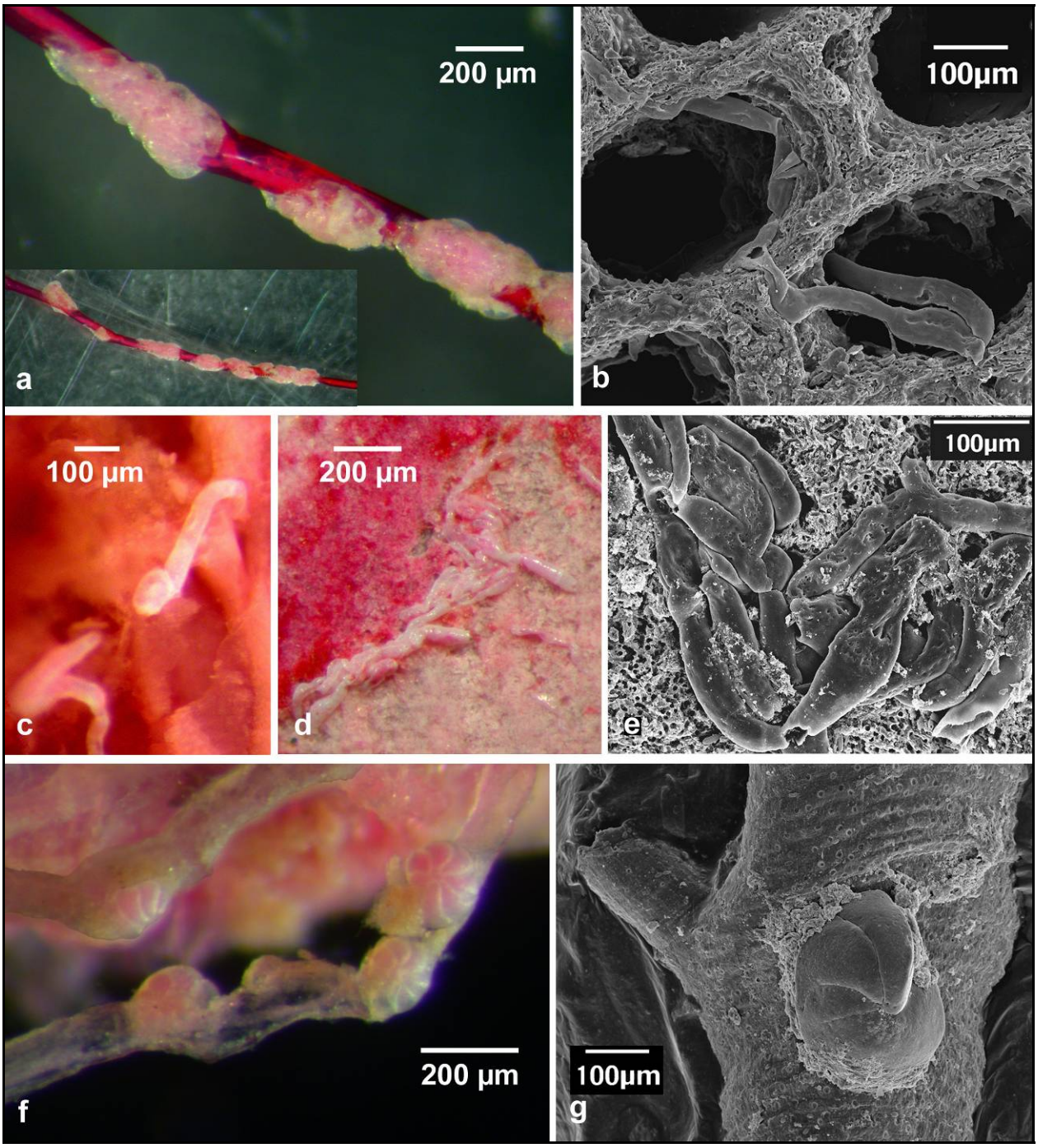


Figure V-3. Attached calcareous species; a, c, d, f, digital photomicrographs, others scanning electron micrographs. a (including inset), *Acervulina* sp. (GI 42C-E20A); b-e, *Calcituba polymorpha* (sensu Arnold; GI82A-W0A); f, g, *Cibicides lobatulus* (f: GI 42C-E20A, g: GI 95A-W20A).

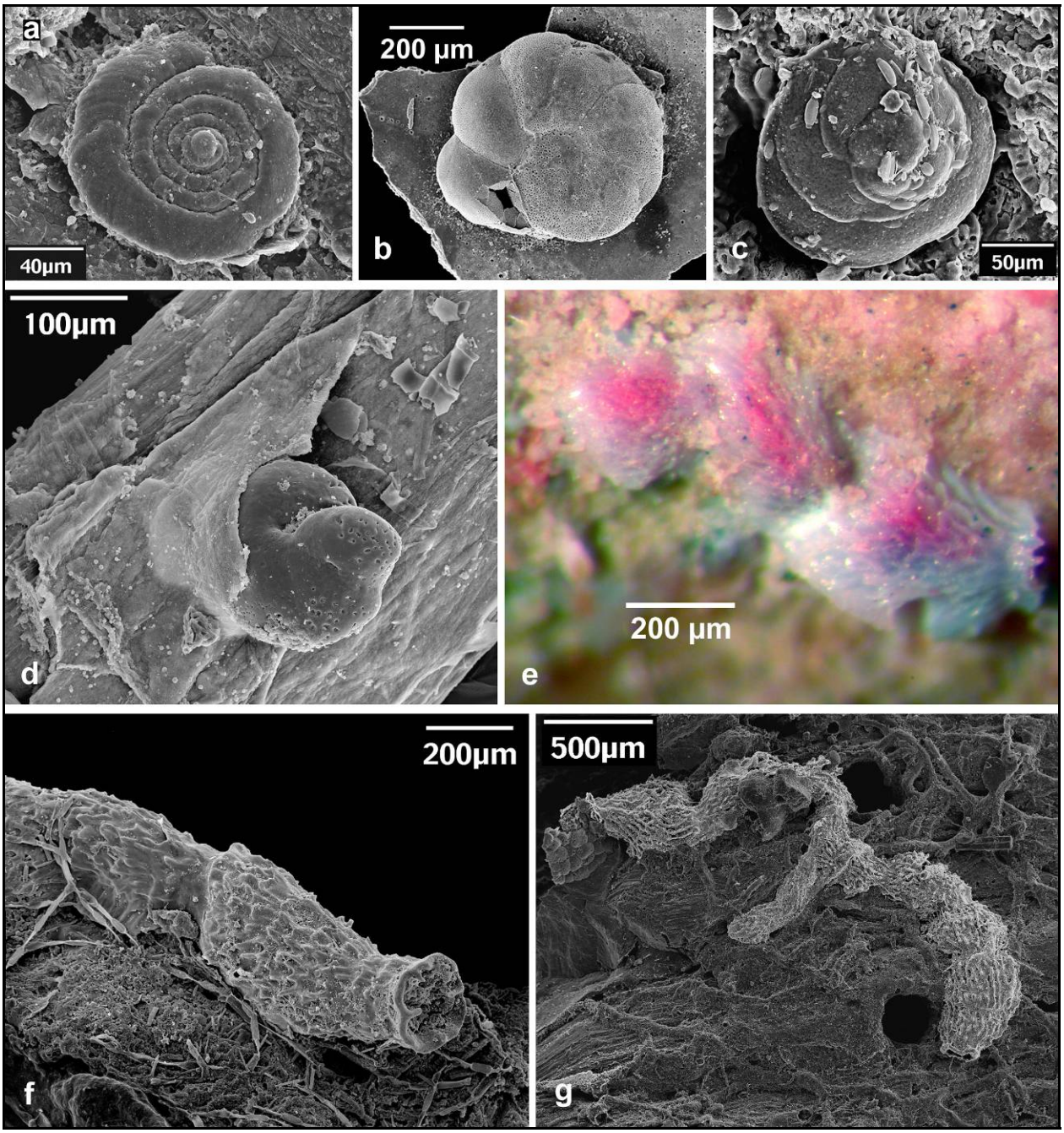


Figure V-4. **Attached calcareous species (contd.); e, digital photomicrograph, others scanning electron micrographs. a, *Cornuspira involvens* (GI 95A-W10A); b*, Hanzawaia concentrica* (ST 67H bottom); c, *Neoconorbina* sp. (GI 95A-W20B); d, *Melonis* sp. (GI 95A-W10A); e-g, *Nodobacularia bonairensis* (e, f: GI 82A-W20A, g: GI 95A-W10A).**

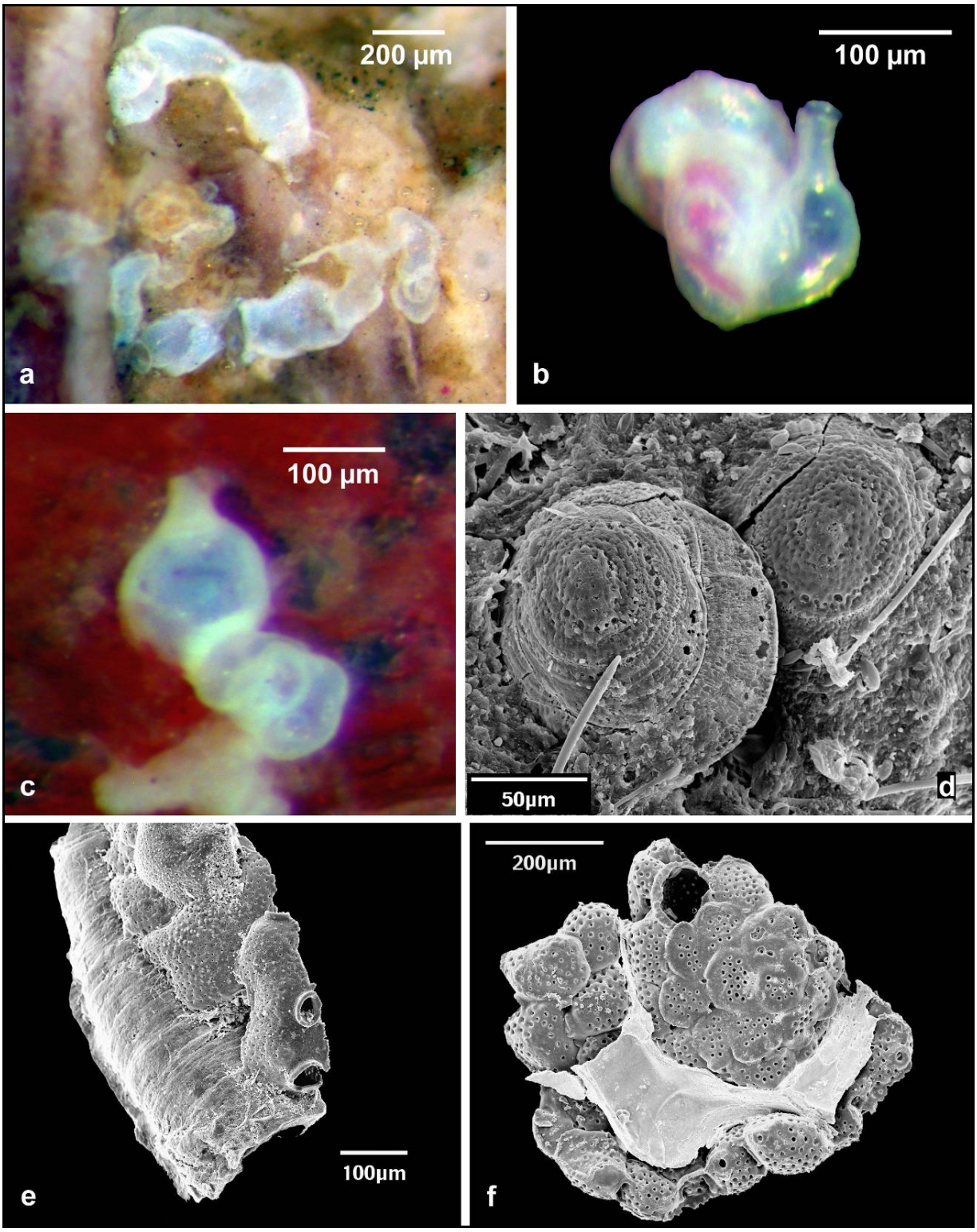


Figure V-5. Attached calcareous species (contd.); a-c, digital photomicrographs, others scanning electron micrographs. a, *Nubeculinita* sp. cf. *N. inhaerens* (ST 67H-W20A); b, c, *Parrina bradyi* (b, detached specimen) (GI 42C-E20A); d, *Patellina corrugata* (ST 67H-E10B); e, f, *Planorbulina mediterranensis* (e: GI 82A-W10B, f: GI 82A-W0A).

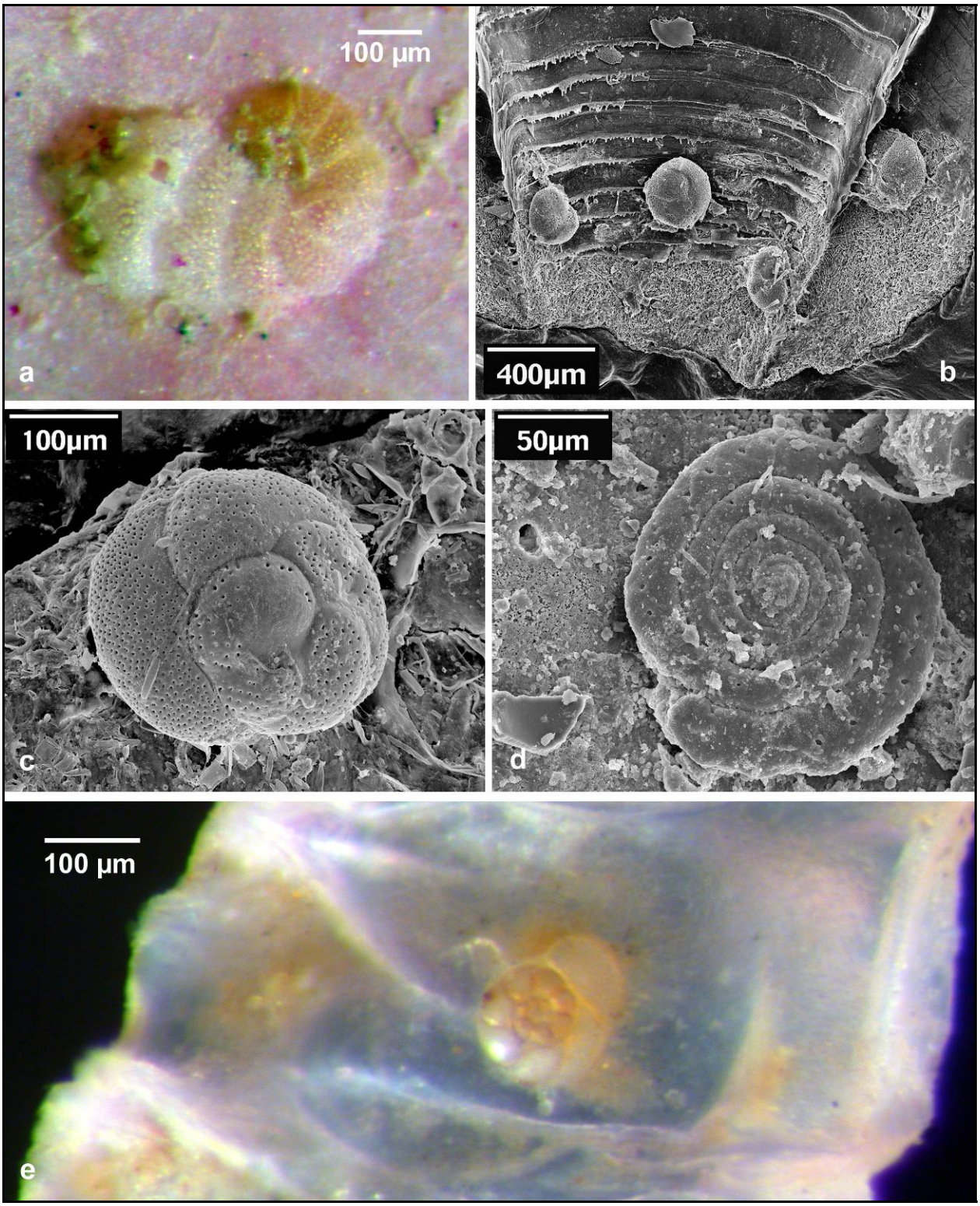


Figure V-6. Attached calcareous species (contd.); a, e, digital photomicrographs, others scanning electron micrographs. a, *Rectocibicides* sp. cf. *R. miocenicus* (ST 67H-W20A); b, c, *Rosalina globularis* (GI 95A-W0A); d, *Spirillina vivipara* (GI 95A-W10A); e, *Rosalina* sp. (GI 42C-E20A).

Mexico. Our samples do not contain *Amphistegina*, the most typical reefal foraminifer of the Caribbean biogeographic province (including the Flower Garden Banks), but whether this genus can occur as a "displaced" taxon on oil and gas platforms (i.e., at "some distance from their usual home range"), as is the case with several reef-type invertebrates (Lewbel et al., 1987), is an open question.

Biotechnology Potential

Agglutinated foraminiferal species hold promise as a source of bioadhesives for biotechnological and biomedical applications, because they can secrete and then harden adhesive organic compounds in an aqueous medium (Bowser et al., 1992). So far, only an Antarctic species, *Astrammina rara*, chosen on the basis of its relatively large size (average ~3 mm diameter) and shallow-water habitat (depth ~25 m, which facilitates harvesting) has been studied for this purpose (e.g., Bowser et al., 1992; Bowser and Bernhard, 1993). The species uses tensile cables of a bioadhesive to bind coarse sand-sized particles (>300 μm) and construct a shell. The chemistry of this bioadhesive has not been fully investigated, but SEM studies indicate the presence of at least two proteinaceous components (fiber and resin). In this context, a task of the MMS/CMI biotic survey of oil and gas platforms was a search for agglutinated foraminiferal species. Both attached species (listed earlier) and free-moving agglutinated species (e.g., *Bigenerina irregularis*, *Spiroplectammina* sp.) were found on the platforms, but not high densities of relatively large specimens (>1 mm) that could be exploited to extract bioadhesives.

CONCLUSIONS

1. The foraminiferal community of Gulf of Mexico platforms includes a great variety of species, of both agglutinated and calcareous wall structures.
2. Many attached species of platforms are rare or absent in adjacent seafloor sediment.
3. No species with biotechnology potential could be identified. Platform related foraminiferans with potential as a source for bioadhesives remains but was not further investigated.

LITERATURE CITED

Arnold, Z.M. 1967. Biological observations on the foraminifer *Calcituba polymorpha* Roboz. Archiv für Protistenkunde 110:280–304.

Bowser, S.S., and J.M. Bernhard. 1993. Structure, bioadhesive distribution and elastic properties of the agglutinated test of *Astrammina rara* (Protozoa: Foraminiferida). Journal of Eukaryotic Microbiology 40:121–131.

Bowser, S.S., J.M. Bernhard, and S.P. Alexander. 1992. Test morphogenesis and bioadhesives in a giant Antarctic foraminifer. Antarctic Journal of the United States 26:165–167.

Brunner, C.A., and P.E. Biscaye. 1997. Storm-driven transport of foraminifers from the shelf to the upper slope, southern Middle Atlantic Bight. Continental Shelf Research 17:491–508.

Gallaway, B.J., and G.S. Lewbel. 1982. The ecology of petroleum platforms in the northwestern Gulf of Mexico: A community profile. U.S. Fish and Wildlife Service, Office of Biological Services, Washington, D.C. FWS/OBS-82/27. Bureau of Land Management, Gulf of Mexico OCS Regional Office, Open-File Report 82-03, xiv + 92 pp.

Goldstein, S.T. 1999. Foraminifera: A biological overview. In: B.K. Sen Gupta (ed.). *Modern Foraminifera*. Kluwer, Dordrecht, pp. 37–55.

Hallock, P. 1999. Symbiont-bearing Foraminifera. In: B.K. Sen Gupta (ed.). *Modern Foraminifera*. Kluwer, Dordrecht, pp. 123–139.

Lewbel, G.S., R.L. Howard, and B.J. Gallaway. 1987. Zonation of dominant fouling organisms on northern Gulf of Mexico platforms. Marine Environmental Research 21:199–224.

Poag, C.W. 1982. Environmental implications of test-to-substrate attachment among some modern sublittoral foraminifera. Geological Society of America Bulletin 93:252–268.

Poag, C.W., and R.C. Tresslar. 1981. Living foraminifers of West Flower Garden Bank, northernmost coral reef in the Gulf of Mexico. Micropaleontology 27:31–70.

Rabalais, N.N., R.E. Turner, and W.J. Wiseman. 2002. Hypoxia in the Gulf of Mexico, a.k.a. "The Dead Zone." Annual Review of Ecology and Systematics 33:235–263.

Rose, P.R., and B. Lidz. 1977. Diagnostic foraminiferal assemblages of shallow-water modern environments: South Florida and the Bahamas. Sedimenta VI, Univ. Miami, 55 pp.

Sen Gupta, B.K. 1999. Foraminifera in marginal marine environments. In: B.K. Sen Gupta (ed.). *Modern Foraminifera*. Dordrecht, The Netherlands: Kluwer Academic Publishers. Pp. 141–159.

VI. BRYOZOANS

Geographical Variation in Chemical Defenses in the Byozoan *Bugula neritina:* Cryptic Species, Cryptic Endosymbionts

Tamara M. McGovern[3] and Michael F. Hellberg

Department of Biological Sciences
Louisiana State University

INTRODUCTION

Natural marine products may eventually provide many useful medicines, however to date the only marine compound to enter phase II clinical trials is bryostatin 1. Bryostatin 1 was initially isolated from the bryozoan *Bugula neritina*, along with other members of a pyran ring family of compounds now termed bryostatins. Bryostatin 1 combats the growth of cultured cancer cells (Hornung et al., 1992) and has shown some promise aiding patients suffering from non-Hodgkin's lymphoma and lymphocytic leukemia (Varterasian et al., 2000) by promoting lymphocyte survival during therapy via action on the protein kinase C signal transduction pathway (see references in Davidson et al., 2001).

Bryostatin 1 has yet to be synthesized in the lab. Isolating gram quantities of bryostatin 1 requires over 10,000 gallons for wet bryozoans as starting material (Schaufelberger et al., 1991), the equivalent of perhaps millions of colonies. Aside from the direct impact that collecting such quantities of *B. neritina* from natural populations, the collateral damage done to other benthic species could be very great. Large sources of *B. neritina* that could be harvested from artificial substrate thus might serve a medical need while sparing natural communities from damage.

Bugula neritina is a common member of temperate fouling communities worldwide. Along the Atlantic and Gulf coasts of North America, *B. neritina* is common on natural and artificial hard substrates (Osman 1977). Significantly, *B. neritina* has been reported as a dominant member of the fouling communities on the legs of shelf oil and gas platforms in the Gulf of Mexico (Gallaway et al., 1981). Whether these platforms could serve as a source of bryostatin 1, however, remains unknown because recent studies reveal that individuals identified morphologically as *B. neritina* actually belong to at least two cryptic species, only one of which harbors the symbiont that produces bryostatin 1 (Davidson and Haygood 1999). Furthermore, if bioactive compounds like bryostatins are produced to defend against predators, then populations might be expected to vary latitudinally in chemical defenses (Harvell et al., 1993) in association with geographic changes in predation rates (Vermeij, 1978, Bertness et al., 1981, Lankford et al., 2001). Along the Atlantic coast of North America, the Cape Hatteras region separates southern waters, where predation pressure is high, from comparatively low predation to the north (Vermeij,1978; Bertness et al., 1981; Lankford et al., 2001). Chemical defenses in several marine animals are known to vary to either side of Cape Hatteras (Stachowicz & Hay, 2000; Sotka & Hay, 2002).

[3] Currently at Clemson University, Clemson, South Carolina.

Bugula neritina is likewise geographically variable in its palatability to predators. Larvae (Lindquist & Hay, 1996) and adults (Stachowicz & Hay, 1999) of *B. neritina* from North Carolina are chemically defended and unpalatable to fish and crab predators, respectively. In contrast, the larvae of morphologically indistinguishable bryozoans from Delaware, also identified as *B. neritina*, are palatable to the same fish that avoid larvae from North Carolina (Lopanik et al., 2004). The larvae of *B. neritina* are large, conspicuous and nutrient rich (Wendt, 2000). They are released during the day (Lindquist & Hay, 1996), swim slowly (Wendt 2000), and spend little time in the plankton (Keough & Chernoff 1987; Wendt 2000). Lindquist & Hay (1996) argued that such larvae may face especially strong selective pressure for defensive adaptations because of their association with the benthos where predation is great. Unlike the crustaceans mentioned above, larvae, particularly large nonfeeding larvae of taxa such as *B. neritina*, tend to defend themselves chemically via the internal production of secondary compounds (Lindquist et al., 1992; Lindquist & Hay, 1996). Bryozoans have proven rich sources of novel organic compounds, several with potential pharmaceutical values (Pettit, 1991), as have many other sessile marine invertebrates (including sponges, gorgonians and tunicates; Faulkner, 2002). Interestingly, these compounds often appear to be produced not by the animal itself, but rather by bacterial endosymbionts (Lee et al., 2001). The larvae of *B. neritina* harbor such bacterial symbionts (Woollacott 1981; Haygood & Davidson 1997).

Davidson et al. (2001) have confirmed previous suspicions (Anthoni et al., 1990) that bryostatins are produced by an endosymbiotic γ-proteobacteria, which they have identified genetically as *Endobugula sertula* (Davidson & Haygood, 1999). The array of bryostatins produced by *B. neritina* varies among populations (Pettit, 1991). Davidson & Haygood (1999) surveyed genetic variation within *B .neritina* along its Pacific range in California. They found two cryptic species of *B. neritina* that harbored different strains of *E. sertula*. The different strains of symbionts produced different bryostatins. Davidson & Haygood (1999) suggested (but did not demonstrate) that the suite of bryostatins produced by each endosymbiont may determine the predators to which its animal host is susceptible. If so, or if endosymbionts produce defensive compounds other than bryostatins, then bryozoan hosts and their bacterial endosymbionts may co-evolve in response to geographical variation in predation pressure.

In this study, we genetically examined whether the *Bugula neritina* growing on shelf oil and gas platforms in the Gulf of Mexico might serve as a source of the putative chemotheraputic agent bryostatin 1. This question was addressed within a larger study (McGovern & Hellberg 2003) in which we examined genetic differentiation among populations of both *B. neritina* and its bacterial associates from its Atlantic range between the Gulf of Mexico and southern New England. Specifically, we asked (i) whether populations of *B. neritina* from north and south of Cape Hatteras, which differ in their palatability, belong to a single genetically mixed species or constitute genetically distinct cryptic species, and (ii) whether differently defended bryozoan populations harbor genetically distinct endosymbionts.

METHODS

Population Sampling

We sampled 9–11 colonies of *Bugula neritina* from five populations south of Cape Hatteras ('Southern' populations: South Terrebonne platform ST67H, LA (28°48′ N, 90°25′ W); Turkey Point, FL (29°54′ N84°29′ W); Cedar Key, FL (29°08′ N, 83°02′ W); Mosquito Lagoon, FL (28°45′ N, 80°45′ W); Beaufort, NC (32°25′ N,80°40′ W)), including the population from

Beaufort, North Carolina that was shown to be unpalatable to fish predators (Lopanik et al., 2004). (.We also sampled 10 individuals from each of two 'Northern' populations (Indian River, DE (38°35′ N, 75°17′ W); Waterford,CT (41°20′ N, 72°08′ W)), including the Delaware site from which palatable animals were collected (Lopanik et al., 2004). In addition, we sampled two or three colonies of *B. stolonifera* from the same Delaware site where *B. neritina* was collected and from Woods Hole, Massachusetts (Marine Biological Laboratory Supply Center, (41°31′ N, 72°08′ W)), and two colonies of *B. turrita* from Woods Hole.

Genetic Variation within *B. neritina*

We used the polymerase chain reaction (PCR) to amplify a portion of cytochrome oxidase c subunit I (COI) with primers LCO1490 and HCO2198 (Folmer et al., 1994). 624 base pairs (bp) of the resulting COI sequence were aligned by eye; there were no indels or stop codons. COI sequences of both deep- and shallow-water forms of *B. neritina* (Davidson & Haygood,1999; GenBank accession numbers AF061422, AF061424, AF061425, AF061432) were included in the alignment. *Bugula stolonifera* and *B. turrita* were used as outgroups. Phylogenetic trees were constructed using the neighbor joining method in PAUP* (Swofford, 2001) with Kimura two-parameter distances. Maximum parsimony trees were generated using both equal and 8:1 weightings of transversions and transitions. We used Modeltest (Posada & Crandall, 1998) to determine most appropriate model for maximum likelihood analysis. Bayesian posterior probabilities were then generated using MrBayes (Huelsenbeck & Ronquist, 2001).

Genetic Variation of Bacteria Associated with *B. neritina*

To identify the bacterial associates of the different bryozoans, we amplified and sequenced > 1,000 bp of the bacterial gene encoding the small subunit ribosomal RNA (SSU rRNA). SSU amplification was a two-step process that first amplified using general bacterial primers, then targeted the Endobugula symbionts using specific primers (Haygood & Davidson, 1997, Table 1 in McGovern & Hellberg 2003). We obtained sequence from a minimum of three clones per individual bryozoan colony using the amplification primers, cloning vector primers and a combination of internal sequencing primers.

In the SSU alignment, we included all of the sequences we obtained, as well as the *E. sertula* sequences obtained by Haygood & Davidson (1997; GenBank accession numbers AF006606–AF006608) and the four most similar bacterial sequences identified using blast searches: *Oceanopirillum maris* (AB006763, Satomi et al., 1998), *O. multiglobuliferum* (AB006764; Satomi et al., 1998) and two uncultured γ - proteobacteria (AB015541; Li et al., 1999 and AF228694). The two most distant of the sequences obtained from our BLAST search (the two *Oceanopirillum* spp.) were used as outgroups. Equally weighted unrooted neighbor-joining and parsimony trees were generated using PAUP*. We also generated a maximum likelihood tree using a model and settings determined by Modeltest.

RESULTS

Cryptic Species within *Bugula neritina*

COI sequences from *Bugula neritina* sampled from the Gulf of Mexico and from the Atlantic coast south of Cape Hatteras were identical (Figs 2A, 3A in McGovern & Hellberg 2003). These sequences were also identical to those of Davidson & Haygood (1999) from Beaufort, NC over the 618 bp where they aligned, thus falling within their Shallow clade of Californian *B. neritina.*

There was a deep divergence (11.5%), however, between these Southern/Shallow form sequences and those obtained from *B. neritina* populations in Delaware and Connecticut. This Northern Atlantic form of *B. neritina* differs not only from the Shallow/Southern form, but also from two other species of *Bugula* found north of Cape Hatteras, *B. stolonifera* (AY173427) and *B. turrita* (AY173428), and from the Pacific deep water form of *B. neritina* identified by Davidson & Haygood (1999). Qualitatively similar trees were obtained using the parsimony with both transition:transversion ratios and using neighbor-joining methods. These trees placed the Southern/Shallow and deep forms as sister taxa, with the Northern clade falling basal within a monophyletic *B. neritina.* Maximum likelihood trees differed topologically, placing the Northern form as the sister to the deep-water clade.

For the bacterial SSU rRNA sequences, all tree building methods produced trees that were topologically similar with regard to relationships among variants of *Endobugula sertula* and between this symbiont and other γ- proteobacteria (Figs 2B, 3B in McGovern & Hellberg 2003). All bacterial sequences (AY173429–AY173431) obtained from the Southern populations of *B. neritina* are identical. They differ by only 2 bp (0.2%) and 4–5 bp (0.5%) from SSU rRNA sequences reported for Haygood & Davidson's (1997) shallow- and deep-water *E. sertula*, respectively. In contrast, the bacterial sequences obtained from *Bugula* found north of Cape Hatteras (AY173432–AY173454) differ by from 5% to 11% from the *Endobugula* sequences from the Southern populations and by 0% to 10% from each other. The bacterial sequences from the Northern *Bugula* show no apparent correlation with either geography or host phylogeny; bacteria from the three species and the six collecting sites (Woods Hole, Connecticut and Delaware) are interspersed. Whereas all Southern bryozoan colonies were associated with identical bacterial sequences, only two sequences associated with the Northern *B. neritina* and the other *Bugula* species were found more than once.

DISCUSSION

The reciprocal monophyly and high level of genetic divergence between the Southern and Northern populations of *Bugula neritina* indicate that this nominal species is most probably a complex of cryptic species along the east coast of the United States, just as Davidson & Haygood (1999) found for *B. neritina* living along the west coast. We suggest that *B. neritina* as currently recognized in US waters comprises three cryptic species: the deep water Pacific form delineated by Davidson & Haygood (1999), a Shallow/Southern form present both in warmer waters along the Pacific coast and along the Atlantic coast south of Cape Hatteras including the Gulf of Mexico, and a third form present in the Atlantic north of the Cape Hatteras region (our 'Northern' form), identified here for the first time (Figure 2A in McGovern & Hellberg, 2003). Thus, no Atlantic *Bugula* species can serve as a source of bryostatin 1.

The identification of two allopatric cryptic species means that differences in larval palatability between Northern and Southern *B. neritina* (Lopanik et al., 2004) do not result from intraspecific responses to geographical variation in predation pressure, as suggested for other species (Sotka et al., 1999; Stachowicz & Hay, 2000; Sotka & Hay, 2002.). Instead, our results indicate that the geographical differences in larval (and perhaps adult) palatability within *B. neritina* result from the presence of two cryptic and allopatric species, each with different defensive capabilities. Other instances of apparent intraspecific variation in morphology and behavior have likewise been found to be the result of grouping together morphologically similar cryptic species (Knowlton et al., 1992; Rowan et al., 1997; reviewed in Shaw, 2001). We cannot eliminate the possibility that past adaptation to differing predation pressures initiated the

differentiation of the two cryptic species, but at present the evidence suggests that these are fully isolated species, and current local adaptation need not be invoked.

The Shallow/Southern species of *B. neritina* has a widespread distribution despite the low dispersal potential of larvae (Keough & Chernoff, 1987; Wendt, 2000), probably reflecting both anthropogenic transport (Carlton & Geller, 1993) and this species' success as a fouling organism. The absence of intraspecific variation seen here is consistent with the possibility that both the Southern/Shallow and Northern *Bugula* species may have been introduced to the ranges studied here. In the face of this demonstrated colonization ability, the failure of the Southern/Shallow species to establish populations north of the Cape Hatteras area, and the failure of the Northern species to establish populations further south, suggests that ongoing ecological forces may act to restrict range expansion of both cryptic species . These factors may include different abiotic tolerances for the two bryozoan species, perhaps for temperature (Davidson & Haygood, 1999). However, differences between the two species in their associations with bacteria may provide a better-supported explanation.

Our results demonstrate that the Northern and Southern species of *B. neritina* differ in their associations with γ- proteobacteria. The Southern *B. neritina* populations appear to have an endosymbiotic relationship with a single species of γ-proteobacteria: *Endobugula sertula*. In contrast, in the Northern populations there was no evidence of a close (or indeed any) symbiotic relationship between *B. neritina* and *E. sertula* or any other γ-proteobacteria.

The suite of bryostatins produced by *E. sertula* may determine the predators to which its bryozoan host is susceptible (Davidson & Haygood, 1999; Davidson et al., 2001). Because the Northern form of *B. neritina* lacks *E. sertula*, their larvae should also lack both bryostatins and any defensive capabilities these compounds confer, capabilities that should be present in larvae of the Southern form. Consistent with this reasoning, the larvae of the Northern form of *B. neritina* are palatable to fish predators whereas the Southern form is unpalatable (Lopanik et al., 2004). Bryostatins were not specifically isolated and tested for their deterrent properties (N. Lopanik, personal communication), but even if the bryostatins are not the source of deterrence, the correlation of deterrence with the presence of *E. sertula* suggests a role for the *Bugula–Endobugula* symbiosis in the response to predators.

CONCLUSIONS

Genetic markers have allowed us to investigate the source of geographical variation in predator resistance within Atlantic populations of the bryozoan *B. neritina* (Lopanik et al., 2004). Using mitochondrial COI sequences, we found that this geographical variation in chemical defense does not result from the local adaptation of a single widespread species to different predation regimes. Rather, the populations surveyed by Lopanik et al. (2004) belong to two genetically differentiated cryptic species: one northern, one southern. Using bacterial small ribosomal subunit sequences, we found that the southern bryozoan species is engaged in a symbiotic relationship with a bacterium (*Endobugula sertula*) known to produce compounds (bryostatins) that may serve in the host's chemical defense. The northern species showed no evidence of such an association. The Gulf of Mexico species also does not produce Bryostatin 1. The ability to identify genetically both cryptic species of *Bugula neritina* and their bacterial associates has allowed us to reject a hypothesis of intraspecific geographical variation in chemical defenses. Instead, the pattern of chemical defenses, and possibly the geographical range of the undefended species, may be determined by species-specific patterns of endosymbiosis.

ACKNOWLEDGMENTS

Specimens were graciously provided by Greg Boland, MMS, Kevin Krajick, Nicole Lopanik, Frank Maturo of the University of Florida Marine Laboratory, Melanie Mead, Barbara Shoplock, Jay Stachowicz, Cheryl Swanson, Jeff Terwin, Linda Walters and the Woods Hole Oceanographic Institute.

REFERENCES

Anthoni, U., P.H. Neilson, M. Perieira, and C. Christophersen. 1990. Bryozoan secondary metabolites: a chemotaxonomical challenge. Comp. Biochem. Physiol. B. 96:431–437.

Bertness, M.D., S.D. Garrity, and S.C. Levings. 1981. Predation pressure and gastropod foraging: a tropical-temperate comparison. Evolution 35:995–1007.

Carlton, J.T. and J.B. Geller. 1993. Ecological roulette: the global transport of nonindigenous marine organisms. Science 261:78–82.

Davidson, S.K. and M.G. Haygood. 1999. Identification of sibling species of the bryozoan *B.neritina* that produce different anticancer bryostatins and harbor distinct strains of the bacterial symbiont 'Candidatus EndoBugula sertula.' Biol. Bull. 196:273–280.

Davidson, S.K., S.W. Allen, G.E. Lim, C.M. Anderson, and M.G. Haygood. 2001. Evidence for the biosynthesis of bryostatins by the bacterial symbiont 'Candidatus EndoBugula sertula' of the bryozoan *B. neritina*. Appl. Env. Microbiol. 67:4531–4537.

Faulkner, D.J. 2002. Marine natural products. Nat. Prod. Rep. 19:1–48.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3:294–299.

Gallaway, B.J., L.R. Martin, R.I. Howard, G.S. Boland, and G.D. Dennis. 1981. Effects on artificial reef and demersal fish and microcrustacean communities. In: Middleditch B.S., ed. Environmental effects of offshore oil production. The Buccaneer Gas and Oil Field Study. Marine Sciences 14:237-293.

Harvell, C.D., W. Fenical, V. Roussis, J.L. Ruesink, C.C. Griggs, and C.H. Greene. 1993. Local and geographic variation in the defensive chemistry of a West Indian gorgonian coral (*Briareum asbestinum*). Mar. Ecol. Prog. Ser. 93:165–173.

Haygood, M.G. and S.K. Davidson. 1997. Small-subunit rRNA and in situ hybridization with oligonucleotides specific for the bacterial symbionts in the larvae of the bryozoan *B. neritina* and proposal of 'Candidatus EndoBugula sertula.' Appl. Env. Microbiol. 63:4612–4616.

Hornung, R.L., J.W. Pearson, M. Beckwith, and D.L. Longo. 1992. Preclinical evaluation of bryostatin as an anticancer agent against several murine tumor-cell lines—in vitro versus in vivo activity. Cancer Res. 52:101-107.

Huelsenbeck, J,P, and R. Ronquist. 2001. MR-BAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Keough, M.J., and H. Chernoff. 1987. Dispersal and population variation in the bryozoan *B. neritina*. Ecology 68:199–210.

Knowlton, N., E. Weil, L.A. Weigt, and H.M. Guzman. 1992. Sibling species in Montastraea-annularis, coral bleaching, and the coral climate record. Science 255:330–333.

Lankford, T.S., Jr., J.M. Billerbeck, and D.O. Conover. 2001. Evolution of intrinsic growth and energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia menidia*. Evolution 55:1873–1881.

Lee, Y.K., J.H. Lee, and H.K. Lee. 2001. Microbial symbiosis in marine sponges. J. Microbiol 39:254–264.

Li, L., C. Kato, and K. Horikoshi. 1999. Microbial diversity in sediments collected f the deepest cold-seep area, the Japan Trench. *Marine Biotechnol.* 1:391–400.

Lindquist, N., and M.E. Hay. 1996. Palatability and chemical defense of marine invertebrate larvae. Ecol. Monogr. 66:431–450.

Lindquist, N., M.E. Hay, and W. Fenical. 1992. Defense of ascidians and their conspicuous larvae: adult vs. larval chemical defenses. Ecol. Monogr. 62:547–568.

Lopanik, N., Lindquist N, Targett N (2004) Potent cytotoxins produced by a bacterial symbiont protect host larvae from predation. Oecologia 139:131-139.

McGovern, T.M., and M.E. Hellberg. 2003. Cryptic species, cryptic endosymbionts, and geographical variation in chemical defenses in the bryozoan *Bugula neritina*. Mol. Ecol. 12:1207-1215.

Osman, R.W. 1977. Establishment and development of a marine epifaunal community. Ecol. Monogr. 47:37-64.

Pettit, G.R. 1991. The bryostatins. Prog. Chem. Org. Nat. Prod. 57:153–195.

Posada, D., and K.A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

Rowan, R., N. Knowlton, A. Baker, and J. Jara. 1997. Landscape ecology of algal symbionts creates variation in episodes of coral bleaching. Nature 388:265–269.

Satomi, M., B. Kimura, M. Hayashi, Y. Shouzen, M. Okuzumi, and T. Fujii. 1998. *Marinospirillum* gen. nov., with descriptions of *Marinospirillum megaterium* sp. nov., isolated from kusaya gravy, and transfer of *Oceanospirillum minutulum* to *Marinospirillum minutulum* comb. nov. Int. J. Syst. Bact. 48:341–1348.

Schaufelberger, D.E., M.P. Koleck, J.A. Beutler, A.M. Vatakis, A.B. Alvarado, P. Andrews, L.V. Marzo, G.M. Muschik, J. Roach, J.T. Ross, W.B. Lebherz, M.P. Reeves, R.M. Eberwein, L.L. Rodgers, and A.J. Shaw. 2001. Biogeographic patterns and cryptic speciation in bryophytes. J. Biogeography 28:253-261.

Sotka, E.E., and M.E. Hay. 2002. Geographic variation among herbivore populations in tolerance for a chemically-rich seaweed. Ecology 83:2721-2735.

Sotka, E.E., M.E. Hay, and J.D. Thomas. 1999. Host-plant specialization by a non-herbivorous amphipod: advantages for the amphipod and costs for the seaweed. Oecologia 118:471-482.

Stachowicz, J.J., and M.E. Hay. 2000. Geographic variation in camouflage specialization by a decorator crab. American Naturalist 156:59-71.

Swofford, D.L. 2001. Phylogenetic analysis using parsimony. Version 4. Sunderland, MA: Sinauer Associates.

Varterasian, M.L., R.M. Mohammad, M.S. Shurafa, K. Hulburd, P.A. Pemberton, D.H. Rodriguez, V. Spadoni, D.S. Eilender, A. Murgo, N. Wall, M. Dan, and A.M. Al-Katib. 2000. Phase II trial of bryostatin 1 in patients with relapsed low-grade non-Hodgkin's lymphoma and chronic lymphocytic leukemia. Clin. Cancer Res. 6:825-828.

Vermeij, G.J. 1978. Biogeography and Adaptation: Patterns of Marine Life. Cambridge, MA: Harvard University Press.

Wendt, D.E. 2000. Energetics of larval swimming and metamorphosis in four species of *Bugula* (Bryozoa). Biological Bulletin 198:356-356.

Woolacott, R.M. 1981. Association of bacteria with bryozoan larvae. Mar. Biol. 65: 155-158.

VII. MOLLUSCS

Mollsca from Oil and Gas Platforms on the Louisiana Continental Shelf: A Biotechnology Survey

Laurie C. Anderson
Dept. of Geology & Geophysics
Louisiana State University

INTRODUCTION

Previous Work

Gallaway and Lewbel (1982) considered offshore platforms to represent a new biological habitat for the northwestern Gulf of Mexico, and a habitat characterized by distinct faunal successions. They recognized three faunal groupings by water depth: coastal (1-30 m), offshore (30-60 m), and blue water (>60 m) (Gallaway et al., 1981; Gallaway and Lewbel, 1982). Changes in faunal composition are apparently influenced by onshore-offshore trends in turbidity; seasonal variation of temperature, salinity, dissolved oxygen, and primary productivity; and exposure to Caribbean water masses (Gallaway et al., 1981; Gallaway and Lewbel, 1982).

Coastal platforms in the western Gulf of Mexico are dominated by barnacles on the shallower portions of platforms, although bivalves can be common on deeper portions of these platforms (at 10 m) (Gallaway et al., 1981; Gallaway and Lewbel, 1982). Bivalves previously reported from Louisiana coastal platforms include several oyster species (*Crassostrea virginica, Ostrea equestris, Lopha frons*, and *Hyotissa thomasi* (10 m) and *Chama macrophylla* (1-30 m). In Texas, *Anadara transversa* (3-12 m), *Chama macrophylla*, *Diplothura smithyi* (21.5 m), and *Isognomon bicolor* (to 12 m) are reported (Fotheringham, 1981; Gallaway and Lewbel, 1982). *Anadara transversa* is, however, an infaunal byssate bivalve, and it is possible that this reported occurrence is a misidentification of another (epifaunal) arcid species.

Bivalves make up the largest portion of the biomass on offshore platforms in the western Gulf of Mexico (Gallaway et al., 1981; Gallaway and Lewbel, 1982). Bivalves reported include *Isognomon* (dominant at 1 m), *Hyotissa thomasi* (dominant from 10-20 m), *Chama macrophylla* (1-20 m), and *Arca imbricata* (1-10 m). Blue water platforms have a lower biomass than those in shallow water, and bivalves are abundant at greater depths on these platforms (Gallaway and Lewbel, 1982).

Bysssate bivalves were included in this study for the biotechnological potential of the bioadhesive used by the byssus.. Byssal bioadhesives provide the mollusc a strong and durable adhesion to underwater surfaces. These non-toxic, biodegradable and non-polluting adhesive proteins have many potential uses including medical and dental adhesives and fillers (Rzepecki and Waite, 1995)

METHODS

Sample Preparation

Samples of molluscs were frozen in the field. Each sample was thawed in the lab and preserved in 95% ethanol. Large pieces (ca. > 4 mm) were stained with Rose Bengal and retained in ethanol. Finer sediment and biota were screened using 63 u, 0.5 mm, 1 mm, and 2 mm sieves, stained with Rose Bengal and preserved in ethanol. Mollusks were identified from the > 2 mm fraction of samples. To compare sampling levels, differences in the number of replicates per sampling level had to be taken into account. However, because the "A" replicate of most sampling levels had a larger volume of biotic material, one replicate per level was not chosen at random. Instead, abundance data per level was averaged across replicates.

RESULTS

The platforms sampled hosted a low diversity molluscan assemblage dominated by byssate bivalves (**Figures VII-1** and **VII-2**). Twenty-seven bivalve and gastropod species were identified, with *Isognomon bicolor* and *Barbatia candida* making up about 90% of the total assemblage. Changes in relative abundance of taxa among platforms (**Figure VII-1**) were in part caused by uneven sampling at the 0-m level. No 0-m samples were collected at ST23 and only one sample was collected at ST67H and GI42C (**Table II-2**). These platforms had a lower relative abundance of *I. bicolor*, a species that is most abundant at very shallow depths (**Fig**ure **2**, and Galloway and Lewbel, 1982). The high dominance of *I. bicolor* at GI82A and GI95A is a result of the large numbers of *I. bicolor* in comparison to numbers of other taxa found in the single replicates taken at 0-m at these platforms.

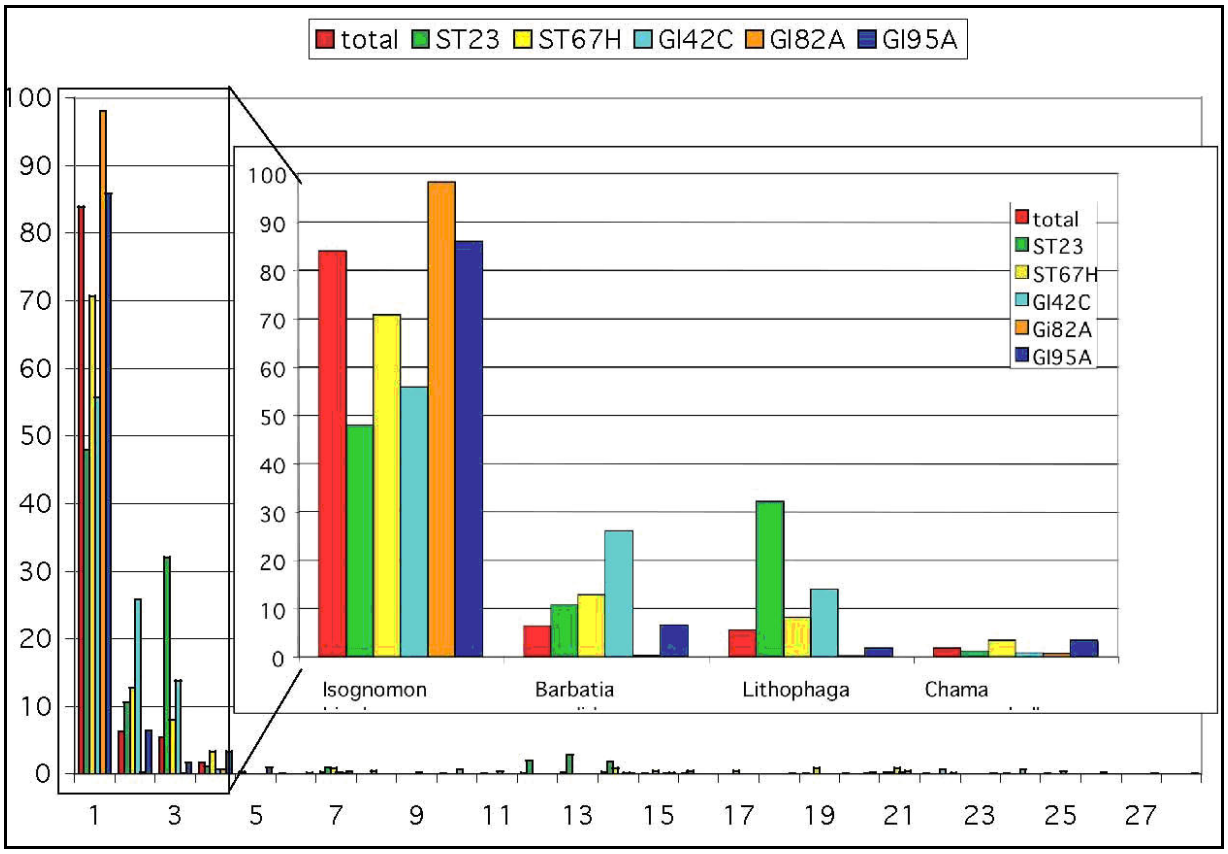


Figure VII-1. Relative abundance of molluscan species from each platform and for all platforms combined (labeled total)

A similar pattern of change in relative abundance with sample depth can be detected for all platforms (**Figure VII-2**). *Isognomon bicolor* dominated (nearly 100%) of 0-m samples. This species also dominated 10-m samples (> 60%), although *Barbatia candida* was also common (nearly 20%) at this level. *Lithophaga* spp. were rare in 0-m samples, but the abundance of these boring bivalves increased with depth, and they were especially common at platform ST 23. *Chama macrophylla* was the fourth most abundant species identified, and although individuals were not numerically abundant, the valves of this species from both live and dead individuals formed an important substrate for other members of the encrusting community.

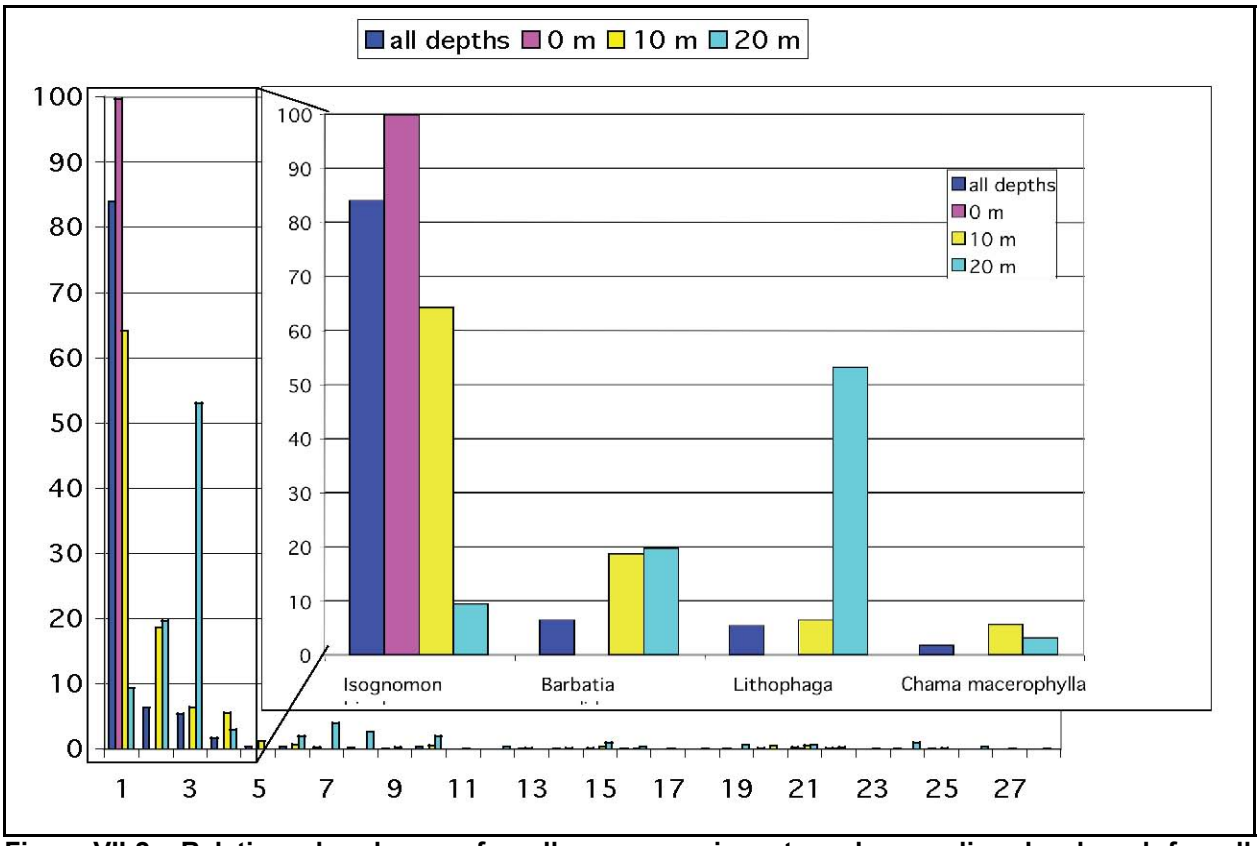


Figure VII-2. Relative abundance of molluscan species at each sampling level and for all sampling levels combined (all depths).

Bivalve composition was similar to that previously reported from oil and gas platforms on the Louisiana and east Texas continental shelf (e.g., Fotheringham, 1981; Gallaway and Lewbel, 1982), where *Isognomon* was most common from 0-12 m; bivalves of the Family Arcidae (arcids) were common from 3-12 m; and *Chama macerophylla* was reported between 1 and 20 m. However, previous reports indicate that oysters were common several meters below the water surface on near shore platforms (Gallaway and Lwebel, 1982). In the platforms examined for this study, three species of oysters were identified (*Ostrea equestris*, *Crassostrea virginica*, and *Lopha frons*) but none were numerically abundant.

The bivalve assemblages identified in this study resembled assemblages found on shorelines characterized by hard substrates in Texas and Mexico (Britton and Morton, 1989). Communities found on naturally hard shorelines in Texas and Mexico contain *I. bicolor* in areas exposed to wave energy and *Barbatia candida* in sheltered areas. *Isognomon bicolor* is also common in the midlittoral zone of jetties and other artificial shoreline structures.

CONCLUSIONS

Potential Significance of Platform Molluscs to Biotechnology

The mollusks collected in this study with the greatest biotechnology potential include the byssate bivalves *Isognomon* and *Barbatia* that produce bioadhesives. The byssus is a bundle of proteinaceous threads secreted by some bivalves that attaches to a substrate by an adhesive plaque (Rzepecki and Waite, 1991; Burzio et al., 1997). These adhesives are of biotechnological

interest because they provide strong, durable adhesion to wet surfaces (Rzepeckiand Waite 1991, 1995). In addition, some of the proteins in the adhesive can chelate metal ions (Martell, 1982; Deming, 1999). The most widely studied byssal protein is mussel adhesive protein (MAP) from *Mytilus edulis*. This compound is used as an attachment factor for cells and tissues in culture (Waite, 1991; Deming, 1999); as an immobilization agent for antigens, antibiotics, and enzymes (Burzio et al., 1997); and as an anticorrosive coating for metals and metal sequestering reagent (Burzio et al., 1997; Rzepecki and Waite, 1995). Additional potential uses are as medical and dental adhesives and fillers; microencapsulating agents; as sizing agents for textiles; and water-resistant inks (Rzepecki and Waite, 1995; Burzio et al., 1997).

Byssal composition is highly variable among taxa (Waite, 1983). Therefore, an examination of byssate bivalves other than *M. edulis* and including those taxa (*I. bicolor* and *B. candida)* found in abundance in this study on Louisiana platforms may lead to the extraction of a new compound with similar or new applications.

LITERATURE CITED

Britton, J.C., and Morton, B. 1989. Shore Ecology of the Gulf of Mexico. The University of Texas Press, Austin, TX. 387 pp.

Burzio, L., Burzio, V., Silva, T., Burzio, L. and Pardo, J. 1997. Environmental bioadhesion: themes and applications. Current Opinion in Biotechnology, 8: 309-312. Deming, T. (1999). Mussel byssus and biomolecular materials. Current Opinion in Chemical Biology 3:100-105.

Fotheringham, N. 1981. Observations on the effects of oil field structures on their bioticenvironment: platform fouling community. In: Middleditch, B.S., ed. Environmental effects of offshore oil production. The Buccaneer Gas and Oil Field Study. Marine Science 14:179-208.

Gallaway, B. and G. Lewbel. 1982. The ecology of petroleum platforms in the northwestern Gulf of Mexico: a community profile. U.S. Fish and Wildlife Service, Office of Biological Services, Washington, D.C. FWS/OBS-82-27. Bureau of Land Management, Gulf of Mexico OCS Regional Office, Open-File Report, 82-03, 92 pp.

Gallaway, B.J., M.F. Johnson, L.R. Martin, F.J. Margraf, G.S. Lewbel, R.L. Howard, and G.S. Bowland. 1981. The artificial reef studies. Vol 2. In: Bedinger, C.A., and L.Z. Kirby, eds. Ecological investigations of petroleum production platforms in the central Gulf of Mexico. SWRI Project 01-5245. U.S. Dept. of the Interior, Bureau of Land Management, New Orleans, LA. 199 pp.

Martell, A.E. 1982. Stability constants of metal-ion complexes. Suppl. No. 1, Part II, Special Publication no. 25. London: The Chemical Society.

Rzepecki, L., and J. Waite. 1991. DOPA proteins: versatile varnishes and adhesives from marine fauna. Bioorganic Marine Chemistry, 4: 119-148.

Rzepecki, L. and Waite, J. 1995. Wrestling the muscle from mussel beards: research and applications. Molecular Marine Biology and Biotechnology 4:313-322.

Waite, J. 1983. Quinone-tanned scleroproteins. In: Hochachka, P.W., ed. The Mollusca, Vol.1, Metabolic Biochemistry and Molecular Biomechanics. New York: Academic Press. Pp. 467-504

Waite, J. 1991. Mussel beards: a coming of age. Chemistry and Industry, 17: 607.

VIII. GENETIC VARIATION OF MOLLUSCS

Genetic variation of *Barbatia candida* (Arcidae; Bivalvia) Populations From Oil Platforms in the Gulf of Mexico

Christopher S. Gregg and David W. Foltz

**Department of Biological Sciences,
Louisiana State University**

INTRODUCTION

Offshore oil and gas platforms represent a unique, man-made hard substrate for fouling organisms in the northern Gulf of Mexico (GOM). Oil and gas platforms in the northern GOM extend from coastal (shore to > 27 m depth), to offshore (27 to 64 m), to blue water (> 64 m) placing them in different water masses that are subject to different hydrodynamic regimes (Gallaway et al., 1981). Consequently, the larvae that colonize the platforms may originate from different sources and dispersal among platforms may be limited. Therefore, the fouling organisms inhabiting platforms in different zones may be cryptic species or exhibit population structure due to a lack of gene flow. Molecular analyses of genetic structure within some nominal species representing diverse marine taxa have revealed heterogeneity suggestive of interspecific differences (Knowlton, 1993; 2000). To date, there have been few studies investigating the taxonomic diversity of the fouling organisms inhabiting oil and gas platforms (e.g., Howard et al., 1980; Gallaway et al., 1981; Gallaway and Lewbel, 1982) and all have relied on morphological identifications. Prior to the present group of studies, none have explored the genetic variability of these organisms in order to confirm their taxonomic status or to evaluate their population structure; however, there have been two studies that examined the genetic diversity of harpacticoid copepods inhabiting the sediments around GOM oil platforms (Street and Montagna, 1996; Gregg et al., 2006).

Taxonomic and genetic heterogeneity of marine organisms must be anticipated prior to surveys for biotechnologically useful molecules and studies of ecological processes. The purpose of the present study was to examine the genetic variability of the bivalve community associated with northern GOM platforms in order to (1) determine if there are cryptic species and (2) examine the genetic variation within and among platforms. To do this, we examined the variability in the mitochondrial cytochrome c oxidase subunit I (COX I) gene. Mitochondrial genes have been used extensively in studies of bivalves to discriminate cryptic species (e.g., O'Foighil et al., 1996; Peek et al., 1997) and to determine population structure (e.g., Reeb and Avise, 1990).

METHODS

Collection of Samples

Samples of attached bivalve mollusks were collected as described in **Section II** and stored in 70% ethanol until they were processed for genetic analysis. Processing consisted of dissecting the soft tissues from each individual and storing the tissues in TE buffer (10 mM Tris pH 8.0; 0.1

mM EDTA) at -20°C. The shells were retained as vouchers for further morphological examination.

Genetic Methods

A small (≈ 2 mm^2) piece of soft tissue was removed from each bivalve. DNA was extracted from this tissue using the CTAB protocol described by Winnepenninckx et al. (1993). A 720-bp portion of the COX I gene was amplified by the polymerase chain reaction (PCR) using the universal primers of Folmer et al. (1994). The PCR was carried out by adding 1 µl of the DNA extract to 5 µl GeneReleaser (BioVentures, Inc.) in a 0.5-µl tube, then incubating the mixture in a thermal cycler using the profile recommended by the manufacturer. After incubation, a PCR master mix was added to each tube. Reactions were done at either a 25 or 50 µl final volume, and the final concentrations of the reagents were as follows: 1X Promega Reaction Buffer A; 2.5 mM MgCl2; 0.2 mM total dNTPs; 1 µM of both forward and reverse primers; 2 units Promega Taq polymerase. The PCR was done in a Perkin-Elmer DNA Thermal cycler under the following conditions: 1 cycle of 95°C for 3 min; 40 cycles of 95°C for 30 sec, 47°C for 45 sec, and 74°C for 1 min; 74°C for 7 min. Three µl of each PCR product was electrophoresed into a 2% agarose gel, stained with ethidium bromide, and viewed by UV transillumination.

The genetic variability of the COX I gene was determined by cutting with restriction enzymes and DNA sequencing. After the PCR, a portion of the amplicon was digested with the restriction enzyme *Mse*I (recognition sequence AATT; New England Biolabs) according to the manufacturer's protocol. The restriction fragments were then electrophoresed on 2% agarose gels, stained with ethidium bromide, and viewed by UV transillumination. DNA sequences were then obtained from randomly chosen individuals from within each restriction profile. The PCR products were purified using Exo-SAP IT (USB) following the manufacturer's protocols, then sequenced using the ABI-Prism Big-Dye cycle sequencing chemistry (Applied Biosystems). The sequencing reactions were scaled down to 10-µl reaction volumes, as described by Rocha-Olivares et al. (2001), and run on an ABI-377 Gene Analyzer (Applied Biosystems).

Data Analysis

The raw DNA sequence data were proofread and assembled into contigs using Sequencher 4.0 (Gene Codes). Previously published sequence data from bivalves in the family Arcidae (Marko 2002; Marko and Moran 2002) were downloaded from the GenBank database (http://www.ncbi.nlm.nih.gov/). The processed sequence data were then aligned using ClustalX (Thompson et al., 1997). Phylogenetic analysis was performed in MEGA 2.0 (Kumar et al., 2001) using the Neighbor-joining method (Saitou and Nei 1987) for tree construction with the Kimuratwo-parameter model (Kimura 1980) of DNA evolution. The reliability of the phylogenetic tree was determined by the nonparametric bootstrap test (Felsenstein 1985). Frequencies of the different COX I restriction profiles within and among platforms were used to determine the population structure by an Analysis of Molecular Variance (AMOVA, Excoffier et al., 1992) using Arlequin 2.0 (Schneider et al., 2000). There were two levels in the hierarchical design of the AMOVA including variability among platforms and the variability within platforms.

RESULTS

Species abundance and diversity are shown in **Figure VIII-1** for each of the five sampled platforms. Further details are provided by Anderson ('Mollusca from Oil and Gas Platforms on the Louisiana Continental Shelf: A Biotechnology Survey') in **Section VII**. RFLP analysis of the COX-I gene was done for 195 *Barbatia candida* (**Table VIII-1**).

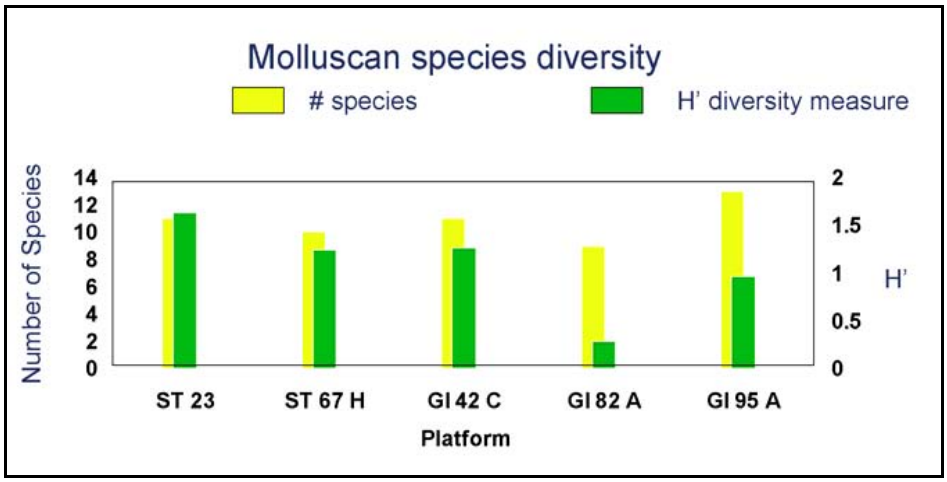


Figure VIII-1. Number of species collected and species diversity.

Table VIII-1

Numbers of *Barbatia candida* Sampled from Five Platforms for RFLP Analysis

Platform	Depth (m)	RFLP Haplotype				Total
		A	B	C	D	
GI 42 C	31	17	37	1	1	56
GI 82 A	52	2	4	0	0	6
ST 67 H	19	6	14	2	1	23
ST 23	15	8	20	3	0	31
GI 95 A	61	19	60	0	0	79
Total		52	135	6	2	195

Twenty DNA sequences from six species within the family Arcidae were downloaded from the GenBank database for comparison with 21 DNA sequences collected in the present study. These species included *Barbatia gradata, B. domingensis, B. reeveana, B. candida, Arca mutabilis*, and *A. imbricata*. Each species formed a separate clade with high (96100%) bootstrap support (**Figure VIII-2**). The 21 DNA sequences from the present study all grouped within the same clade as previously-published B. candida sequences, suggesting that there are no cryptic species among the arcid molluscs from the platforms examined. The average uncorrected distance among sequences from the present study was 0.006 with a maximum of 0.0075. Maximum p-distance = 1.7% (3.4%); overall mean p-distance = 0.6% (0.8%). Genetic distance is consistent with intraspecific variation, i.e., no cryptic species.

Restriction fragment profiles were analyzed to determine the level of genetic variation present and to find evidence of cryptic species. The restriction fragment length analysis revealed

the presence of four haplotypes at the three platforms, which will be referred to as A - D. DNA sequences have been obtained from individuals consisting of haplotypes A and B (A - Bc 9, 10, 19, 28; B – Bc 13, 32, 35; see Figure VIII-2) confirming that they are located within the *B. candida* clade and are likely the same species; however, no sequence data have yet been collected to confirm the phylogenetic status of haplotypes C and D. For the following analysis, we assumed that these 2 haplotypes also fell within the *B. candida* lineage. No significant population structure was detected using AMOVA (FST = 0.014; p>0.05) with essentially all the variation found among individuals within platforms compared to between platforms (**Table VIII-2**). The negative estimate of FST is likely a result of the true value being close to zero. Taxonomic units labeled Bc # (in **Table VIII-2**) are from the present study. *Barbatia candida* sequences are monophyletic.

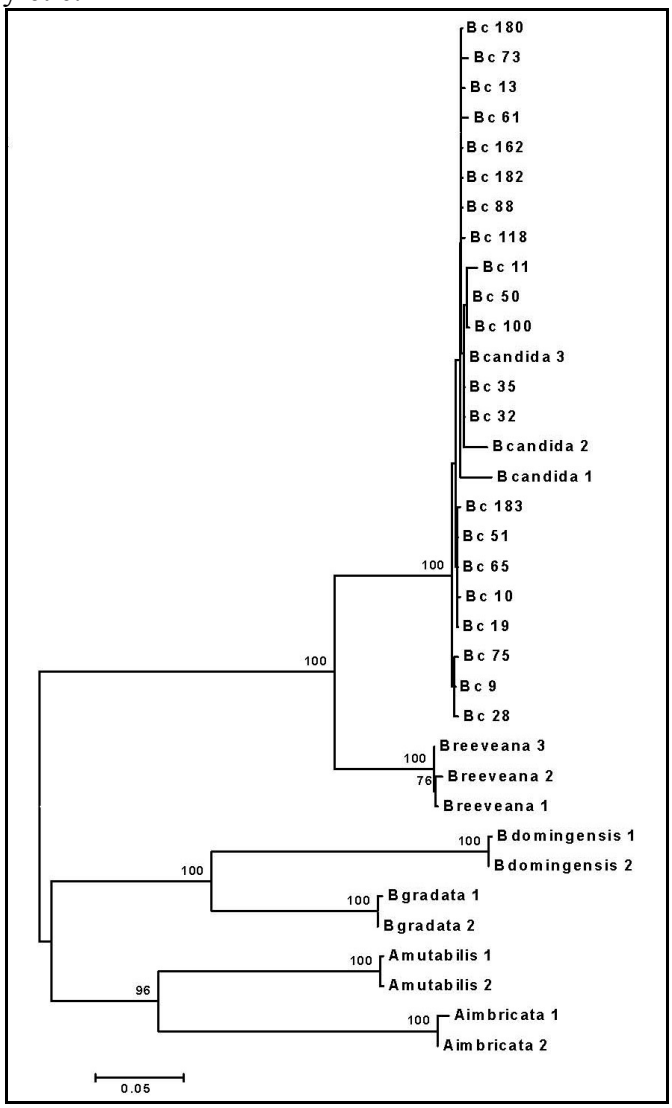


Figure VIII-2. Comparison of mitochondrial COX I DNA sequence data with previously published data on Arcidae (Marko & Moran, 2002; Marko, 2002).

Table VIII-2

Results of AMOVA Analysis Showing Genetic Variation of *Barbatia candida* within and among Five Platforms In The Northern Gulf of Mexico.

Source of Variation	Variance Component	% of Variation
Among Platforms	-0.0058	-1.45
Within Platforms	0.2456	101.45

DISCUSSION

The genetic analysis of the Arcidae collected from five platforms in the northern Gulf of Mexico has shown no evidence of cryptic species or population structure. The RFLP and sequence data collected both indicate that all of the individuals are *Barbatia candida*. The Arcidae are known to show significant intraspecific morphological variation among populations in different habitats suggesting the potential for cryptic species (Marko and Jackson, 2001). Collecting data for more populations from differing habitats (i.e., coastal, offshore, and blue water) may reveal greater taxonomic heterogeneity within this group. The RFLP analysis suggested that there was no structuring of the arcid populations inhabiting the five sampled platforms. Bivalves in the family Arcidae disperse by means of planktotrophic larvae that may remain in the plankton for up to one to two weeks (Chanley and Andrews, 1971). Given the closeness of the platforms, for which samples have been studied thus far, it is not surprising that gene flow among them is sufficient to homogenize the populations. Nonetheless, genetic discontinuities have been observed among geographically close bivalve populations when hydrographic barriers exist (e.g., Reeb and Avise, 1990). Analysis of samples from platforms located in differing hydrographic regimes may reveal restricted gene flow among populations.

ACKNOWLEDGMENTS

We thank Lorene Smith for processing samples on board ship, Nannette Crochet for assistance with DNA sequencing and Jennifer Kiger, Collin Richie and Kinda Seaton for assistance in the laboratory.

LITERATURE CITED

Chanley, P., and J.D. Andrews. 1971. Aids for the identification of bivalve larvae of Virginia. Malacologia 11(1):45-120.

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Folmer, O., M. Black, R. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotech. 3(5):294-299.

Gallaway, B.J., and G.S. Lewbel. 1982. The ecology of petroleum platforms in the northwestern Gulf of Mexico: a community profile. U.S. Fish and Wildlife Service, Office of Biological Services, Washington D.C. FWS/OBS-82/27. Bureau of Land Management, Gulf of Mexico OCS Regional Office, Open File Report 82-03. xiv + 92 pp.

Gallaway, B.J., M.F. Johnson, L.R. Martin, F.J. Margraf, G.S. Lewbel, R.L. Howard, and G.S. Bowland. 1981. The artificial reef studies. Vol 2. In: Bedinger, C.A., and L.Z. Kirby, eds. Ecological investigations of petroleum production platforms in the central Gulf of Mexico. SWRI Project 01-5245. Bureau of Land Management, New Orleans OCS, LA. 199 pp.

Gregg, C. S., D. W. Foltz, and J. W. Fleeger. 2006. Chapter 13: Genetic diversity of harpacticoid copepods. In: Continental Shelf Associates, Inc. Effects of Oil and Gas Exploration and Development at Selected Continental Slope Sites in the Gulf of Mexico. Volume II: Technical Report. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2006-045. Pp. 441-457.

Howard, R.L., G.S. Boland, B.J. Gallaway, and G.D. Dennis. 1980. Effects of gas and oil field structures and effluents on fouling community production and function. Vol V. In: Jackson, W.B., and E.P. Wilkens (eds). Environmental assessment of Buccaneer gas and oil fields in the northwestern Gulf of Mexico, 1978–1979. NOAA Technical Memorandum NMFS-SEFC-39, 60p.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120.

Knowlton, N. 1993. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24:189-216.

Knowlton, N. 2000. Molecular genetic analysis of species boundaries in the sea. Hydrobiologia 420:73-90.

Kumar, S., K. Tamura, I.B. Jakobsen, and M. Nei. 2001. MEGA2: Molecular Evolutionary Genetics Analysis software, Arizona State University, Tempe, AZ.

Marko, P.B. 2002. Fossil calibration of molecular clocks and the divergence times of geminate pairs separated by the Isthmus of Panama. Mol. Biol. Evol. 19(11):2005-2021.

Marko, P.B., and J.B.C. Jackson. 2001. Patterns of morphological diversity among and within arcid bivalve species pairs separated by the Isthmus of Panama. J. Paleontol. 75:590-606.

Marko, P.B., and A.L. Moran. 2002. Correlated evolutionary divergence of egg size and a mitochondrial protein across the Isthmus of Panama. Evolution 56(6):1303-1309.

O'Foighil, D., T.J. Hilbish, and R.M. Showman. 1996. Mitochondrial gene variation in Mercenaria clam sibling species reveals a relict secondary contact zone in the western Gulf of Mexico. Mar. Biol. 126(4):675-683.

Peek, A.S., R.G. Gustafson, R.A. Lutz, and R.C. Vrijenhoek. 1997. Evolutionary relationships of deep-sea hydrothermal vent and cold-water seep clams (Bivalvia: Vesicomyidae): results from mitochondrial cytochrome oxidase subunit I. Mar. Biol. 130(2):151-161.

Reeb, C.A., and J.C. Avise. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, Crassostrea virginica. Genetics 124:397-406.

Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406-425.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Street, G.T., and P.A. Montagna. 1996. Loss of genetic diversity in Harpacticoida near offshore platforms. Mar. Biol. 126:271-282.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The ClustalX-Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc. Acids Res. 25:4876-4882.

Winnepenninckx, B., T. Backeljau, and R. De Wachter. 1993. Extraction of high molecular weight DNA from molluscs. Trends Genet. 9:407.

IX. CONCLUSIONS

The Gulf of Mexico has over 3,000 oil and gas platforms that provide hard substrate for the attachment of a large and diverse assemblage of epifaunal or fouling organisms. Many of these organisms have the potential to provide compounds useful in industrial and medical applications. Bioprospecting for new compounds requires preliminary elucidation and clarification of the taxonomy of the organisms present and, in some instances, their chemical ecology. The macrofaunal assemblages unique to the oil and gas platforms of the Gulf are not well known and, in this study, the taxonomy and relative abundance with depth and distance offshore of major taxa of epifaunal organisms were addressed. This study involved investigation of various aspects of five major taxa :

Bacteria
Macroalgae
Foraminifera
Bryozoa *
Molluscs

*The study of the Bryozoa centered on a single genus, *Bugula,* known to be the source of an important chemical, bryostatin, used in cancer teatment.

Conclusions relative to the study of these taxa are as follows.

BACTERIA

The study demonstrated that large numbers of bacterial species, many of which are novel and belong to taxonomic groups known to be of biotechnological importance, were present in the fauna. There are a number of factors that would seem to influence the bacterial load and diversity of the biomatrix recovered from a given location.

Examination of the bacterial species present in the biomatrix revealed that this is a habitat of novel bacterial species that represents a biotechnological and scientific resource waiting to be exploited. Isolation and natural product screening still has its place in biotechnology but the application of whole genome studies to such environments is the future path. The construction of total genome libraries from the biomatrix of oil and gas platforms and their expression will no doubt provide novel bioactivities from both the micro and macro components of these complex systems. This study established that the biomatrix encrusting oil and gas platforms represents a baseline of species and a potential starting point for material for such studies.

MACROALGAE

The total number of macroalgal taxa collected during this study was 24. The best represented group was Rhodophyta (approximately 50% of the taxa). There was a vertical distribution of taxa found on the platforms themselves, and there was also a trend identified for biodiversity and abundance of macroalgae to increase from nearshore to offshore and decrease from intertidal to depth.

In all, seven new taxa were added to the list of macroalgae identified from the platforms and there was one new report of *Antithamnionella breviramosa* from the Gulf of Mexico.

Approximately 50% of the taxa collected are known to have biotechnological potential and approximately 20% of the taxa collected are found only on oil and gas platforms.

FORAMINIFERA

Agglutinated foraminiferal species hold promise as a source of bioadhesives for biotechnological and biomedical applications because they can secrete and then harden adhesive organic compounds in an aqueous medium. The foraminiferal community of Gulf of Mexico oil and gas platforms includes a great variety of species, of both agglutinated and calcareous wall structures. No species with biotechnology potential, however, could be identified in this particular study although further study of the foraminferal fauna may reveal biotechnological potential. Taxonomical work with this group will be necessary prior to identifying their potential contribution to any biotechnological potential.

BRYOZOA

This study found that B. neritina as currently recognized in US waters comprises three cryptic species: the deep water Pacific form, a Shallow/Southern form present both in shallower Pacific waters and along the Atlantic coast south of Cape Hatteras, and a third form present in the Atlantic north of the Cape Hatteras region. The species collected from the Gulf of Mexico platforms does not produce the commercially important bryostatin I although its cytochrome oxidase subunit sequences were identical to those collected from the Atlantic south of Cape Hatteras.

MOLLUSCS

Bivalve composition was similar to that previously reported from platforms on the Louisiana and east Texas continental shelf where *Isognomon* was most common found from 0-12 m. Bivalves of the Family Arcidae (arcids) were common from 3-12 m; and *Chama macerophylla* was reported between 1 and 20 m.

The bivalve assemblages identified in this study resembled assemblages found on shorelines characterized by hard substrates in Texas and Mexico. The byssus are of biotechnological interest because they provide strong, durable adhesion to wet surfaces. In addition, some of the proteins in the adhesive can chelate metal ions.

The most widely studied byssal protein is mussel adhesive protein (MAP) from *Mytilus edulis*. This compound is used as an attachment factor for cells and tissues in culture; as an immobilization agent for antigens, antibiotics, and enzymes; and as an anticorrosive coating for metals and metal sequestering reagent. Additional potential uses are as medical and dental adhesives and fillers; microencapsulating agents; sizing agents for textiles; and water-resistant inks.

Byssal composition is highly variable among taxa. Therefore, an examination of byssate bivalves other than *M. edulis* and including those taxa (*I. bicolor* and *B. candida)* found in abundance in this study on Louisiana platforms may lead to the extraction of a new compound with similar or new applications.

The genetic analysis of the Arcidae collected from five platforms in the northern Gulf of Mexico has shown no evidence of cryptic species or population structure. The RFLP and sequence data collected both indicate that all of the individuals are *Barbatia candida*. The Arcidae are known to show significant intraspecific morphological variation among populations

in different habitats suggesting the potential for cryptic species. Collections from more populations from differing habitats (i.e., coastal, offshore, and blue water) in the Gulf of Mexico may reveal greater taxonomic heterogeneity within this group. Analysis of samples from platforms located in differing hydrographic regimes and more spatially isolated may reveal restricted gene flow among populations.

This study provides a foundation for future analyses of epifauna that may be of biotechnological potential. Organisms may be initially screened for use in medical and industrial applications with little knowledge of their ecology. There is, however, a need for much more information about the taxonomy, seasonality, and ecology of these organisms before any questions can be resolved regarding ways to farm and harvest them economically if they are determined to have medical or industrial applications.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Minerals Revenue Management** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.