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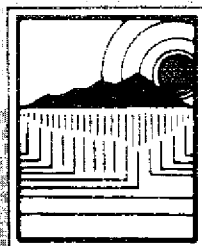
**Reproductive Biology and Ecology of Some
Caribbean Corals**

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Project Number R-101-1-99

Progress Report

PRU-T-99-(4)



Sea Grant College Program

UNIVERSITY OF PUERTO RICO

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ANNUAL PROGRESS REPORT

(May, 2000)

Project: Reproductive Biology and Ecology of Some Caribbean Coral

Project #: R-101-1-99

Funds for first year: \$ 74,796

PI: Ernesto F. Weil

Associate Professor

Depart. Marine Sciences - UPR

Date funds available: April 1999. Total Funds for first year = \$ 75,224

Date project started: April 1999.

1. Introduction

Knowledge of the reproductive biology and the associated processes of dispersal and recruitment is essential for the interpretation of ecological, geographical and evolutionary studies of coral populations. Even though there has been a significant compilation of information on the reproductive modes and patterns of scleractinian corals, this information is mainly for Indo-Pacific species. In the Caribbean there is information on some aspect of the reproduction for 18 of the 60+ species of zooxanthellate corals. In many cases, there are conflicting reports or confusing results. Given that Caribbean reefs are suffering from increasing detrimental conditions, there is an urgent need to increase our knowledge and understanding of the reproductive biology and ecology of the rest of the species. In this study we proposed to gather information on the sexual reproductive pattern and mode of development of up to 24 species not previously studied in detail. This information will include : (a) determination of the sexual pattern (gonochoric or hermaphroditic, and the development mode (spawner or brooder); (b) assessment of the number, sequence and timing of the gametogenetic cycles per year, (c) assessment of the fecundity of each species; (d) to experiment with larvae rearing and recruitment under laboratory conditions, and (e) to train graduate students.

2. Personnel

Two persons were hired to help out in this project, a Research Associate and a graduate student:

A- The Research Associate has been responsible for the following tasks :

- Supervising of all activities in the histology lab at Isla Magueyes which include:
 - . Search and selection of the equipment needed, negotiate with the manufactures and purchase of the equipment needed to fix, process and analyze the histological samples of coral tissues. Maintenance of the equipment.
 - . Selecting, negotiate prices and purchase of all laboratory materials and chemicals needed throughout the project.
 - . Maintaining the inventory of these chemicals and materials up to date and restock what is being used.

- To help in the collection and processing of the samples in the field and then, in the lab.
- To process samples (de-calcification, embedding, cutting and staining) in the laboratory.
- To analyze samples under the microscope with the PI to determine the maturity state of the gametes in the gonads.
- Supervise and train graduate students on all aspects of coral histology techniques and analyses of samples.

B- The graduate student will have some tasks to do and will have the opportunity to develop a Masters dissertation project within the scope of the program. This person responsibilities included:

- To assist the PI in the collection and preservation of the samples in the field
- To assist the Research Associate in the processing and analyses of samples in the laboratory.
- To produce a thesis and at least one per-review article.

3- Laboratory set-up

During the first three months of the project (after the funds were received), all the necessary glassware, chemicals, lab materials, permanent equipment, and field collection and storage materials were purchased. The histology laboratory was setup in the visitors lab of the Department of Marine Sciences at Isla Magueyes. This facility had some counter space, storage space, hot and cold water, a refrigerator and most importantly, an extraction hood. The project provided a long table to increase counter-top space to set up the image analysis system and to provide space for one graduate student. The facility provides enough working space for the Associate Researcher and one graduate student at this moment.

4- Permanent Equipment

Most of the permanent equipment was purchased during this year. Two pieces of permanent equipment remain to be purchased at the beginning of the second year. The following list includes only major permanent equipment purchased:

- 1- Sakura Finetex rotary tissue processor (\$ 8,400.00)
- 2- Sakura Finetex tissue embedding system unit (\$ 6,600.00)
- 3- Ohaus balance (\$ 944.20)

Many other minor pieces of equipment like water baths, hot-plates/magnetic stirrer, hot plates for thin sections, etc. were also purchased. Many other pieces of equipment needed (microtome, dissecting scope, vacuum oven, electronic balance, etc.) to process the samples were provided by the PI and the Department of Marine Sciences. A compound microscope with an image analysis system (digital camera, computer and software) will be purchased at the beginning of the second year.

5- Laboratory materials and expendables

All laboratory materials and chemicals needed for the first year have been purchased. We will keep purchasing on a regular basis as the processing of samples and usage of these products continues. Because the cost of the major pieces of equipment was lower than anticipated, funds from the equipment account were transferred to the materials account to complement the purchase of needed chemicals, laboratory materials, some pieces of equipment like pipettes, cleaning products and office materials. Some computer peripherals needed for the processing and storage of photographs, video clips, data, figures and information were also acquired. Software updates and an image analysis software to do quantitative measurements from the tissue thin sections were purchased. We have had problems with the acquisition of some chemical products, and there have been unexplained, long delays in the delivery of other products important for the staining process. The reorganization of the CID has affected significantly their efficiency in processing the requisitions and purchase orders.

6. Field work materials

All the equipment and materials for the location, measurement, tagging and collection of samples for the first year were purchased. These included measuring tapes, pvc pipes, tools, cable ties, plastic and aluminum tags, flagging tape, re-bars, dive bags, dive knife, galvanized nails, hydraulic drill, high impact drill, nylon cord, video tapes and floppy disks for video camera and digital still camera, etc.

PROGRESS

Starting in February 1999, Monthly tissue samples were collected from at 5-10 colonies of each of a total of twelve (table 1) abundant scleractinian coral species in three reef areas off La Parguera. Tissue samples from two species of *Madracis* were also collected for one month (June-99) in Los Roques, Venezuela. Ten of the twelve species have very little or no information on their gametogenetic cycles and other reproductive characteristics. The other two have only partial information that needs to be complemented.

The total number of tissue samples collected over 13 months amounts to 1177. Of these, 740 samples have been partially or completely processed (fixed-decalcified-embedded-cut-stained) to the final staining process. It is important to clarify that, from each tissue sample, at least ten sets of thin sections (sagittal and transverse sections) are mounted in microscope frosted slides and then stained. These samples will be observed under a compound microscope and an image analysis system to determine the gametogenetic cycle, the sexual patterns, and time and mode of development for the different species. Rough estimations of fecundity will be made from the histological samples, however, once we determine the reproductive cycle and spawning or brooding timing, we will be able to collect spawn from individual cores and determine the fecundity (# eggs/polyp) for the different taxa.

Table 1. Scleractinian coral species sampled during the first year of the project (1999-2000), and the current status of the processing of tissue samples in the laboratory.

Genus / species	Start - sampling	End - sampling	Total months sampled	Total # of Samples	Samples processed
<i>Madracis</i>					
<i>M. decactis</i>	Feb - 00	March - 01	13	100	56
<i>M. mirabilis</i>	Feb - 00	March - 01	13	114	50
<i>M. formosa</i>	Feb - 00	March - 01	13	78	48
<i>M. pharensis</i>	Feb - 00	March - 01	13	122	74
<i>M. senaria</i>	Feb - 00	March - 01	13	65	21
<i>Diploria</i>					
<i>D. clivosa</i>	Feb - 00	March - 01	13	78	78
<i>D. strigosa</i>	Feb - 00	March - 01	13	106	106
<i>D. labyrinthiformis</i>	Feb - 00	March - 01	13	84	84
<i>Stephanocoenia</i>					
<i>S. intersepta</i>	Feb - 00	March - 01	13	102	54
<i>Colpophyllia</i>					
<i>C. natans</i>	Feb - 00	March - 01	13	86	46
<i>Eusmilia</i>					
<i>E. fastigiata</i>	Feb - 00	March - 01	13	124	62
<i>Scolymia</i>					
<i>S. cubensis</i>	Feb - 00	March - 01	13	118	61

A brief check of stained tissue from *Diploria labyrinthiformis* has revealed that this species, contrary to preliminary observations, shows mature eggs and spermaries in the months of February and May. Information from the other two species in this genus indicates that they are mature in the warmer months of the year, August-September. We still need to check the rest of the samples for *D.labyrinthiformis* and those of the other two species of *Diploria*, however. If the temporal difference holds, then, our results will support preliminary evidence that *D. labyrinthiformis* might not belong in the genus *Diploria* and might need reclassification to another or a new genus.

A novel strategy of asexual reproduction in Caribbean corals: Gemmae in *Diploria* and other massive-columnar species

Modular organisms show a wide variety of reproductive strategies. Two sexual reproductive patterns (gonochoric and hermaphroditic) with two modes of development (brooding or spawning) and at least five different mechanisms of asexual reproduction have been reported for scleractinian corals. Corals can reproduce asexually by fragmentation, polyp-bail-out, polyp expulsion, polyp balls and asexual production of larvae. Until recently, the only mechanisms of asexual reproduction reported and/or observed in Atlantic-Caribbean corals were budding, fragmentation and asexual production of larvae.

As a separate research topic, but within the scope of the project, the PI expanded the project to include the study of a novel (never been described) asexual mechanism in massive species of Caribbean corals. Anecdotal observation in the early 70's by JC Lang and P Dustan indicate the presence of these structures in colonies of *Diploria strigosa* and *Dendrogyra cylindrus* in Jamaica (jc Lang, personal communication). Similar observations and the finding of "rolling stones" in *D. strigosa*, were brought to the PI attention by a graduate student of the Department of Marine Sciences, Mr. Austin Bowden, in 1997. Since then, we have observed the gemmae in two other species of *Diploria* and in *Dendrogyra cylindrus*. A pilot project to study the ecological characteristics and implications of this novel asexual mechanism was initiated in La Parguera, Puerto Rico, by the PI and three graduate students of the Department of Marine Sciences. Observation and data were also collected by the PI in other reef areas of the wider Caribbean (Bermuda, Bonaire and Venezuela). This means that this novel asexual strategy has a wide geographic distribution and is not a local phenomenon, therefore, we can speculate that it must have a genetic basis and an evolutionary advantage.

The strategy involves the asexual production (gemination = budding) of small, multicellular bodies which eventually produce skeletal structures (Fig. 1). We selected the term gemma (pl. gemmae = asexual reproductive body) to describe these bodies. A single gemma starts as a soft tissue growth on the sides or on top of the ridges (septa-ambulacra) (Fig.1). As it grows, the soft, pigmented (zooxanthellae), round or oval gemma deposits the hard skeleton (Fig. 2) which is not directly attached to the parent colony. It has an irregular set of trabeculae-like structures at the point of soft-tissue attachment, and well formed, regularly spaced septa on the top. Gemmae remain attached to the parent colony until water movement (surge, currents, wave

action) or weight, force them to brake away. At this point, the gemmae is usually large (7 to 20 mm in length) and heavy and falls to the substrate, where it either form “rolling stones”, reattach to the substrate or die (Fig. 3). Histological sections showed an enlarged mesoglea in the tissue section connecting the gemma to the parental colony.

Quantitative surveys in several reefs in La Parguera an in Venezuela showed that: (1) gemmae are only found on shallow water colonies (down to 4-6 m in well exposed areas); (2) populations of the different *Diploria* species show differences in the relative frequency of colonies with gemmae (11.2 % in *D.labyinthiformis*, 12.7% in *D.strigosa*, and 20.7% in *D.clivosa* in Puerto Rico, and 3 % in *D.clivosa*), and 23.3 % in *D.strigosa*) in Venezuela) (Fig.4); (3) the number of gemmae per colony varied widely from 1 to 27 in Venezuela and 1 to 110 in PR; (4) the maximum gemma size measured was 12 mm; (5) number and size of gemmae was not related to colony size; and (6) gemmae can remain attached to the parent colony for over one year.

Recruitment of slow-growing massive species has been considered solely as the result of sexual reproduction and the rates of recruitment reported have been usually very low. One persistent question is therefore, how populations can persist with such low rates of successful recruitment?. One possible answer to this question is that some massive species produce asexual propagules that get dispersed short distances and attach rapidly to the substrate where they have higher survival rates (larger size) than the small sexual recruit. This asexual reproductive strategy could have evolved in these massive corals as a response to the environmental instability of shallow reef areas. Until recently, only three mechanisms of asexual reproduction have been reported and/or observed in Atlantic-Caribbean corals: budding, fragmentation of mostly branching species and asexual production of larvae (*Tubastrea aurea*). This new mechanisms may not only help to explain the survival of population of species which show low rates of recruitment but, it would explain why some species or genera like *Diploria* are so abundant (dominant) in shallow water habitats. This study will be an important contribution of this project.

The PI plans to continue studying this asexual mechanism to answer some of the other question dealing with the consequences in the genetic composition of shallow water populations of *Diploria*, the rate of gemmae production per colony, and survival rates.

Presentations

An oral presentation of the preliminary results was given at the B 29th Benthic Ecology Meetings in Wilmington, NC, in March of 1999, and a second one with more information on the ecology of the process (copy of abstract included) will be given at the 9th International Coral Reef Symposium to be held in Bali in October of this year. A manuscript will be submitted within the next month to weekly journal Nature.

SUMMARY OF EXPENSES BY CATEGORY (approximated)

<u>Total Budget for First Year</u>		<u>Start</u>	<u>expended</u>
1- Salaries and bonifications personnel	-----	\$ 20,516	21,700
2- Benefits	-----	\$ 3,284	3,500
3- Student stipends	-----	\$ 3,000	3,000
3- Permanent Equipment	-----	\$ 26,493	19,224
5- Materials	-----	\$ 6,535	12,202
6- Indirect cost	-----	\$ 14,598	14,598
Total	-----	\$ 75,224	74,224

Plans for the 200-2001 year of the project

The plans are to finish the processing and analyses of the tissue samples of all the species sampled during the first year (Table 1). During the second year we will add 8 more species, most of them brooders and in the family Mussidae (Table 2) to this study. Collecting started in February 2000 and will continue until March of 2001. Processing and analyses will be implemented as we go along with finishing the samples of 1999-2000.

Table 2. Species and number of months of sampling for the second year.

Genus / Species	Start sampling	End sampling	Total months
<i>Mycetophyllia</i>			
<i>M. ferox</i>	Feb-00	March-01	13
<i>M. aliciae</i>	Feb-00	March-01	13
<i>M. lamarckiana</i>	Feb-00	March-01	13
<i>M. danaana</i>	Feb-00	March-01	13
<i>Isophylastrea rigida</i>	Feb-00	March-01	13
<i>Isophyllia sinuosa</i>	Feb-00	March-01	13
<i>Cladocora arbuscula</i>	Feb-00	March-01	13

Gemma development in *D. strigosa*

Tissue out growths



Calcification & growth

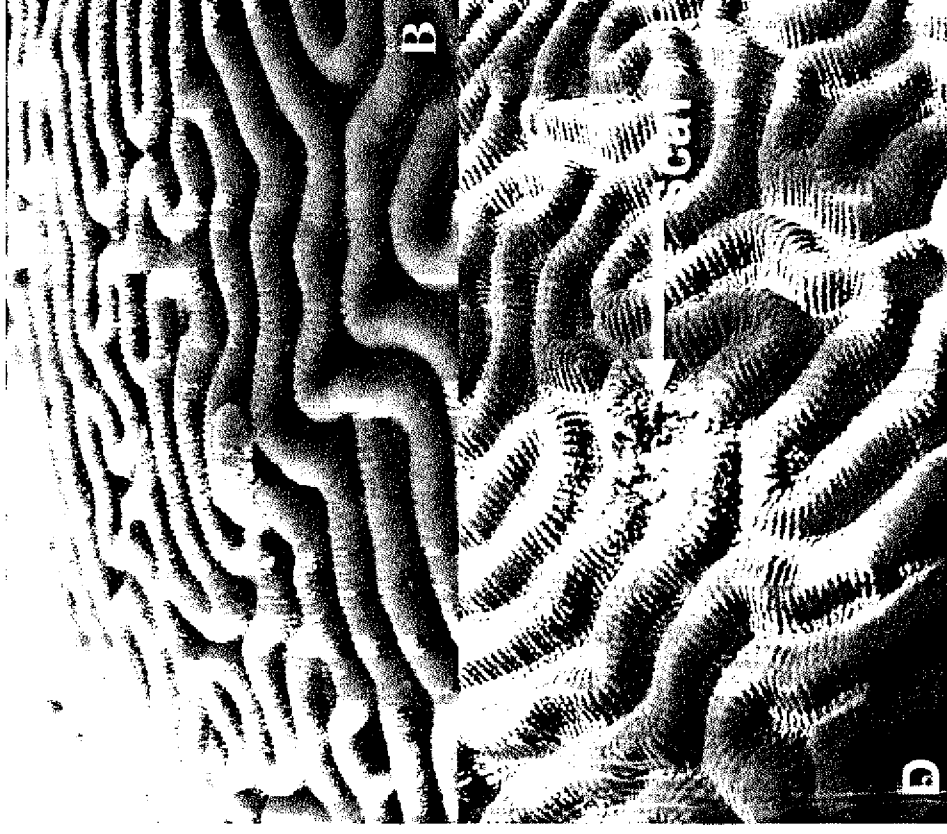


Figure 1. Gemmae development (A) small tissue outgrowths, (B) calcified gemma, (C) large gemma hanging from thinning tissue connection to parent colony and (D) scar left by detached gemma.

Gemmae Skeleton

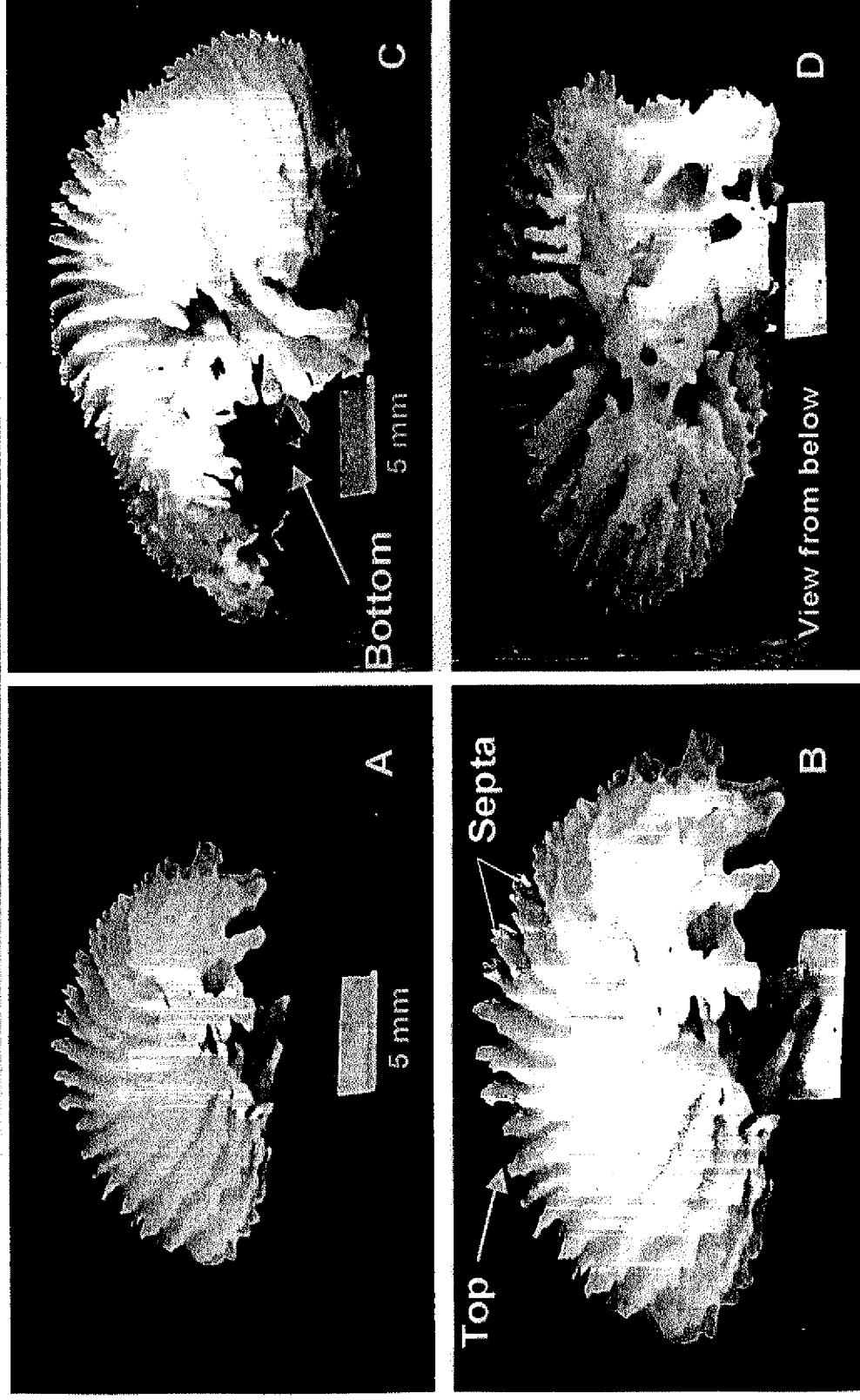


Figure 2. Skeletal structure of *Diploria strigosa* gemma showing the well formed septa (A, B) on the top, and the irregular structure of skeletal components on the bottom (C, D), where the tissue connection with the parental colony is located.

Detached surviving
gemmae

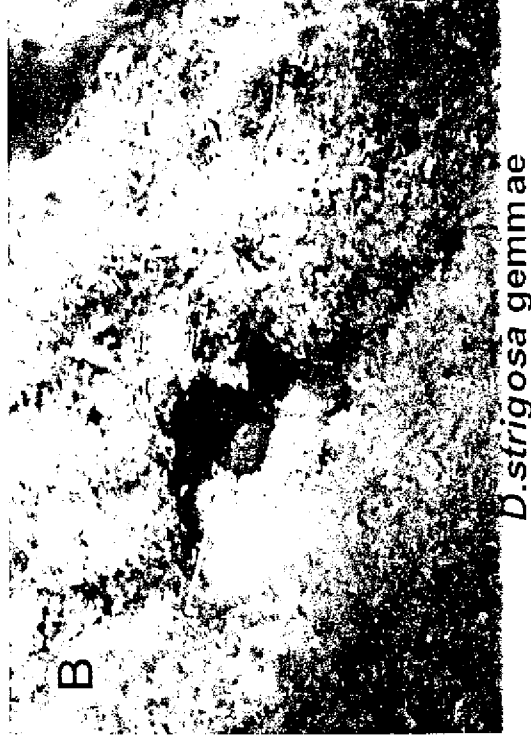
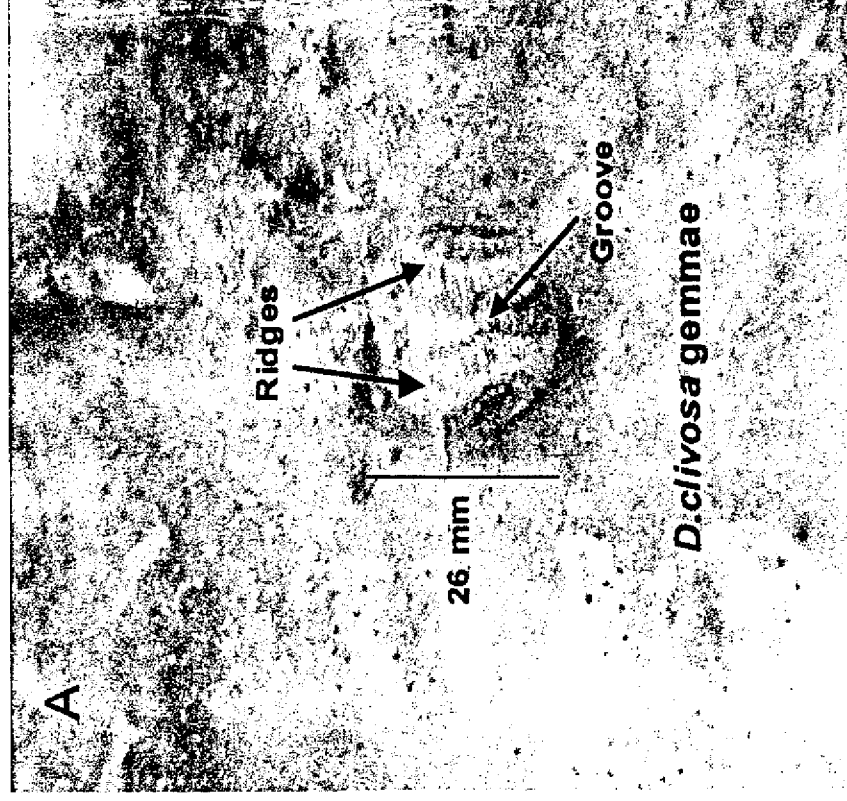


Figure 3. Detached gemmae from *D. clivosa* (A) and *D. strigosa* (B,C) found alive in the the field. (A) is resting crustose algae, (B) is in a crevice and (C) is in the open.

Relative proportion of colonies with gemmae

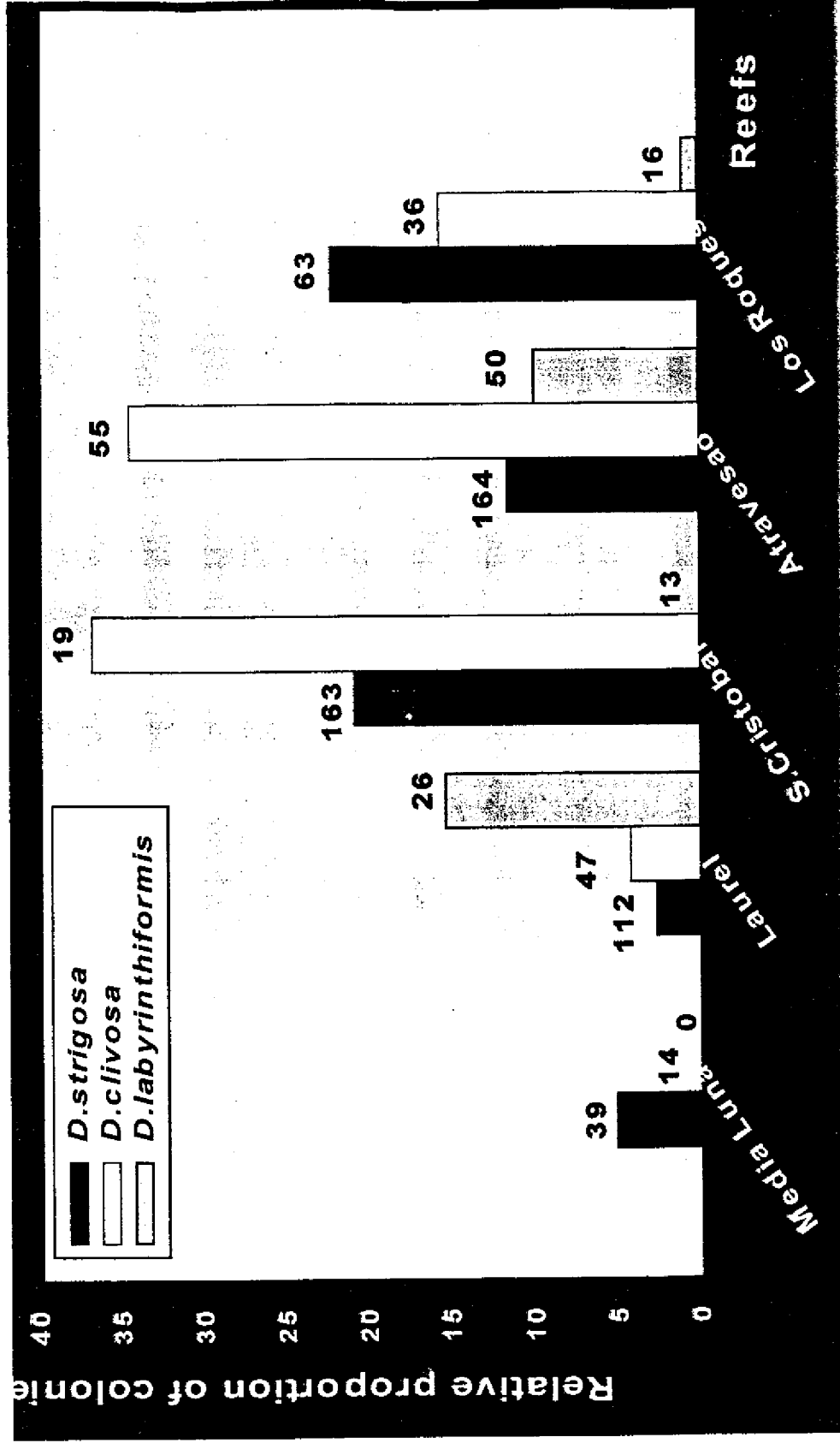


Figure 4. Relative abundance of colonies with gemmae within population of the three different species of *Diploria* in reefs off La Parguera, Puerto Rico, and Los Roques, Venezuela.

