

UNIVERSIDADE ESTADUAL DE CAMPINAS

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**Estrutura de comunidade e variabilidade genética de  
ascídias coloniais do entremarés rochoso**

Dissertação apresentada ao Instituto de Biologia da  
Universidade Estadual de Campinas para obtenção  
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Campinas

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Ao Professor Sérgio de Almeida Rodrigues (*in memoriam*)  
e aos meus queridos pais

Há dois tipos de pessoas: As que fazem as coisas, e as que dizem que fizeram as coisas.

Tente ficar no primeiro tipo.

Há menos competição.

(Indira Ghandi)

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## RESUMO

A diversidade, sazonalidade, distribuição espacial e ocupação de microhabitats por ascídias coloniais do entremarés rochoso foram estudadas nas praias da Baleia e Grande, ambas no Município de São Sebastião, litoral norte do Estado de São Paulo. Os dados foram coletados mensalmente, durante o período de janeiro de 2001 a janeiro de 2003, por meio de sorteio de parcelas de 1m<sup>2</sup>. Trinta e três espécies de ascídias coloniais foram amostradas, sendo a família Didemnidae a mais representativa com dezenove espécies no total, das quais duas são inéditas para a ciência. A Praia Grande apresentou maiores riqueza e diversidade do que a Praia da Baleia, o que é, provavelmente, devido à diferença no hidrodinamismo das duas praias. Tanto a riqueza, quanto a dominância variaram entre as três áreas do entremarés (inferior, mediana e superior) com zonação de espécies apenas na Praia Grande. A variação destes atributos ao longo do ano demonstrou que a taxocenose estudada é mais diversificada e rica durante os meses mais quentes. Face inferior de rocha foi o microhabitat mais utilizado por esse grupo de organismos, devido provavelmente ao comportamento fotofóbico da larva. Simultaneamente, a variabilidade genética da ascídia colonial *Symplegma rubra* proveniente no litoral dos Estados de São Paulo e Rio de Janeiro foi avaliada através do método de isozimas. Foi encontrada alta variabilidade genética e moderada estruturação ( $F_{ST}=0.051$ ) entre as populações distanciadas por até 363Km. Estes resultados contradizem os já descritos na literatura para ascídias coloniais de regiões de clima temperado. A manutenção do fluxo gênico observada reforça a importância de maneiras alternativas para dispersão em grandes distâncias, como o “rafting” (dispersão de colônias por substratos flutuantes), uma vez que ascídias coloniais apresentam larvas lecitotróficas de curta duração no plâncton. Variações observadas na frequência alélica para o loco *Pgi* sugerem efeitos de pressões seletivas relacionados à diferença de temperatura da água do mar entre as áreas estudadas, reforçando a importância deste fator sobre ascídias coloniais.



## ABSTRACT

The diversity, seasonality, spatial distribution and microhabitat occupation by colonial ascidians on a rocky intertidal zone were studied at Praia da Baleia and Praia Grande, both in the São Sebastião district, on the northern coast of São Paulo State. Samples were collected, monthly from January 2001 to January 2003 by raffling 1m<sup>2</sup> quadrats. Thirty-three species of colonial ascidians were identified, with the family Didemnidae being the most represented (19 species, of which two were previously unknown). Praia Grande had a greater richness and diversity than Praia da Baleia, probably because of differences in the hydrodynamic processes at the two beaches. Richness and dominance varied among the three areas of the intertidal zone (low, middle and high), with species zonation occurring only at Praia Grande. Variation in these parameters during the year showed that this taxocoene was more diversified and richer during the warmest months. The undersurface of rocks was the microhabitat most used by these organisms, probably because of the photophobic behavior of the larvae. The genetic variability of the colonial ascidian *Symplegma rubra* found on the coasts of the States of São Paulo and Rio de Janeiro was assessed by allozyme analysis. Extensive genetic variability and a moderate structure ( $F_{ST}=0.051$ ) were observed among populations separated by up to 363 km. These results differed from those for colonial ascidians in temperate areas. The maintenance of gene flow observed here reinforces the importance of alternative ways, such as rafting, for dispersal over great distances, particularly since colonial ascidians have lecithotrophic larvae that spend only a short time in plankton. Variations in the allele frequencies for the locus *Pgi* suggested that were selective pressures related to differences in seawater temperature among the studied areas. This finding reinforced the importance of temperature in determining the distribution of colonial ascidians.

## INTRODUÇÃO GERAL

O conceito de biodiversidade reúne a riqueza de espécies, com a diversidade de habitats e ecossistemas nos quais elas são encontradas. O desenvolvimento do estudo da biodiversidade deve relacionar processos ecológicos com as histórias evolutivas dos organismos que participam destes processos (Neigel, 1999). Desta forma, análises do padrão de distribuição espacial são essenciais para o entendimento do grau de interação dos organismos ou destes com o seu ambiente (Underwood & Chapman, 1996).

Tanto a história e a localização biogeográfica de uma região, como as interações da comunidade, podem influenciar processos populacionais e, desta forma, determinar a distribuição e as abundâncias relativas dos organismos (Ricklefs, 1996). Os tamanhos dessas populações, bem como os padrões de acasalamento, o fluxo gênico, as mutações, a seleção natural e as interações desses processos são os fatores responsáveis pela distribuição e frequência dos genótipos nas populações naturais (Hartl & Clark, 1988).

No caso de animais marinhos de costão rochoso, diferenças ecofisiológicas entre as espécies propiciam diferentes resistências à dessecação, ao impacto de ondas e às variações de salinidade e de temperatura, influenciando, portanto, a fixação das larvas nos substratos e a sobrevivência dos adultos (Nybakken, 1993). Esses fatores, associados à predação e ao fato de o espaço ser geralmente o recurso limitante mais importante no costão (Jackson, 1977), implicam em uma distribuição espacial diferencial dos organismos e conseqüente zonação de espécies neste tipo de ambiente.

A capacidade de dispersão das espécies é um importante fator para a estabilidade e manutenção da biodiversidade (Keough, 1984; Scheltema, 1986). Uma alta capacidade de

dispersão permitirá à espécie ocupar e explorar diferentes habitats, aumentando a sua longevidade pela redução das taxas de extinção em ambientes com grandes variações ambientais (Hansen, 1978). Isto também evita efeitos adversos do endocruzamento, mantendo a continuidade genética entre metapopulações e reduzindo as taxas de especiação (Wilson & Hessler, 1987). Além da influência sobre os processos populacionais, a capacidade de dispersão afeta fortemente a distribuição biogeográfica e batimétrica dos organismos marinhos (Young *et al.*, 1997).

Ascídias (Subfilo Tunicata, Classe Ascidiacea) são invertebrados sésseis, exclusivamente marinhos, (Rodrigues *et al.*, 1998), comuns em todo o mundo. Seus representantes estão distribuídos amplamente, desde águas rasas até grandes profundidades (Rocha, 1988; Rodrigues *et al.*, 1999), onde se prendem a rochas, conchas, talos de algas, pilares de piers, fundos de navios, e algumas vezes são fixados à lama ou areia por meio de filamentos ou de uma haste (Kott, 2001).

Em geral são animais de águas com alta salinidade, entretanto, algumas espécies podem suportar variados graus de diluição (Millar, 1971). Em certos lugares a baixa salinidade impossibilita a reprodução, permitindo apenas a ocorrência de espécies mais resistentes, devido à sensibilidade dos zigotos, embriões ou larvas (Dybern, 1967, 1969), as quais são, em geral, lecitotróficas de curta duração no plâncton (Young *et al.*, 1997; Osman & Whitlatch, 1995).

A influência da luz sobre as larvas de ascídias é bem conhecida, sendo um dos principais fatores controladores da microdistribuição do grupo (Millar, 1971). A escolha dos locais de fixação pelas larvas é o que determina o padrão de distribuição das espécies, podendo a intensidade luminosa influenciar diretamente na seleção de microhabitats (Dybern, 1969). Laubier (1966) *apud* Millar (1971) listou os microhabitats de 21 espécies

de ascídias tendo encontrado apenas três espécies fotofílicas. Já o padrão de distribuição espacial, ou zonação, das espécies de ascídias é determinado, principalmente, pelas diferenças de tolerância à ação das ondas, à exposição ao ar, e ao nível de turbidez da água, sendo que este último fator está diretamente relacionado à disponibilidade de oxigênio e de alimento (Berril, 1932, Young *et al.*, 1997).

A organização colonial, resultante da reprodução assexuada por brotamento, surgiu independentemente várias vezes dentro da Classe Ascidiacea (Millar, 1966; Wada *et al.*, 1992; Stach & Turbeville, 2002). Em geral, os zoóides são muito pequenos, embora a colônia possa atingir um tamanho considerável, muitas vezes de um metro ou mais. Nestes casos os indivíduos produzidos permanecem unidos entre si por estolões ou dentro de uma túnica comum (Rodrigues *et al.*, 1998).

Quase todas ascídias coloniais completam embriogênese internamente e liberam larvas que nadam ativamente permanecendo na coluna d'água, geralmente, não mais que algumas horas antes da fixação no substrato (Millar, 1971), o que resulta numa dispersão espacial restrita, quando comparadas a organismos que apresentem larvas planctotróficas (Grosberg, 1987). Através de experimentos de marcação e recaptura e histocompatibilidade, Grosberg (1987) mostrou que, para a espécie de regiões de clima temperado *Botryllus schlosseri*, a maioria das larvas não dispersa por mais do que poucos metros do seu local de origem. Outros estudos constatam que ascídias apresentam uma tendência à distribuição gregária, seja pela produção de feromônios (Burke, 1986), ou pela baixa mobilidade das larvas (Osman & Whitlatch, 1995).

Considerando que a dispersão de organismos marinhos sésseis ocorre principalmente durante o estágio larval, espera-se que, diante deste contexto, ascídias apresentem alta taxa de endemismo, com espécies populacionalmente estruturadas (Burton,

1983). No entanto vários estudos vêm demonstrando que a forma de desenvolvimento não é o único fator que determina a distância de dispersão e que métodos alternativos de dispersão, como o “rafting” (dispersão de colônias por substratos flutuantes), podem ter importância subestimada na manutenção do fluxo gênico entre populações isoladas (Jokiel, 1984; 1989; Worcester, 1994).

Lambert & Lambert (1998) notificaram uma alta taxa de introdução de espécies exóticas nos portos e marinas da Califórnia. O mesmo foi constatado no Canal de São Sebastião, Estado de São Paulo, por Rodrigues & Rocha (1993), Rocha & Monniot (1995) e Rodrigues *et al.* (1999). Segundo Monniot *et al.* (1991), o aparecimento de espécies exóticas em muitos portos é um fenômeno mundial. Tal fato pode ser explicado pela facilidade de recrutamento e rapidez de crescimento de ascídias em cascos de navios que não tenham sido pintados recentemente (Rodrigues *et al.*, 1999), ampliando os limites de ocorrência das mesmas, em consequência do rápido transporte de navios entre portos do mundo todo (Lambert & Lambert, 1998).

Em regiões de clima temperado, a abundância de larvas de ascídias no plâncton é sazonal, devido à grande sensibilidade das mesmas a baixas temperaturas (Dybern, 1965). Yamaguchi (1975), acompanhando o crescimento de ascídias tanto coloniais como solitárias, verificou um aumento do tempo de duplicação de tamanho com a redução de temperatura.

Devido à maior homogeneidade climática anual dos trópicos, em relação às regiões de clima temperado, é esperado que não haja grandes flutuações na comunidade de ascídias durante o ano. Porém, Rocha (1991) encontrou uma grande variação no número de colônias no litoral do município de São Sebastião (SP) ao longo do ano, dados que, segundo a autora, estão significativamente relacionados com as temperaturas médias mensais.

Resultados preliminares do projeto de iniciação científica de G.M.Dias permitiram verificar que, dentre as espécies estudadas, várias apresentam uma grande redução do tamanho e número de colônias, não sendo encontradas, em alguns casos, durante alguns meses do inverno. O reaparecimento das colônias após este período ainda não é compreendido, podendo ser resultante de novos recrutamentos, de expansão de colônias quiescentes que se mantêm durante o inverno, ou mesmo de ambos os processos. Estudos que compreendam análises da variabilidade genética dessas espécies podem ser de fundamental importância para a elucidação da capacidade de dispersão das mesmas e dos processos que estão envolvidos na sazonalidade deste grupo de organismos.

## OBJETIVOS:

Estudos que procuram entender os padrões de distribuição de ascídias coloniais são raros, e diferenças na fauna destes urocordados em habitats aparentemente similares, ainda não foram totalmente elucidadas. Neste contexto, este projeto teve por objetivos:

- Caracterizar a biodiversidade de ascídias coloniais da região entremarés de substratos consolidados da Praia Grande de São Sebastião, SP.
- Comparar a composição e a diversidade de ascídias coloniais da região entremarés das praias Grande e da Baleia, considerando o padrão de distribuição espacial, a zonation e a seleção de microhabitats das espécies presentes.
- Descrever espécies de ascídias coloniais desconhecidas pela ciência, coletadas em ambas as praias.
- Avaliar a variabilidade genética de *Symplegma rubra*, uma espécie de ascídia colonial, sazonal, abundante ao longo do litoral sudeste do Brasil, com o intuito de entender o grau de estruturação e a intensidade de fluxo gênico entre suas populações.

## **Capítulo 1**

**Estrutura de comunidade de ascídias coloniais do entremarés  
rochoso de São Sebastião, SP**





RUNNING HEAD: Community structure of colonial ascidians

TITLE: Community structure of colonial ascidians from the intertidal zone at São Sebastião, São Paulo State, Brazil.

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## **1.1. ABSTRACT**

The composition, richness and diversity of colonial ascidians in the intertidal zone of two rocky beaches, Praia Grande and Praia da Baleia, at São Sebastião, on the coast of the State of São Paulo, Brazil were assessed by studying the distribution pattern, zonation, seasonality and microhabitat occupation of various species. Monthly sample collections in the intertidal zone of these two beaches showed that ascidians were more abundant and diversified during the warmest seasons. The undersurface and lateral surfaces of rocks were the most used microhabitats, with the differences between the two beaches being related to the hydrodynamic processes at each site. The two rocky shores had high species richness when compared to other studies. Two new species were recorded from these shores, and there were marked changes in the composition of the ascidian fauna on Praia Grande, over a 10-year interval. Significant differences in species richness, diversity and dominance were also recorded between the upper intertidal zone and the middle and lower ones at both sites.

## 1.2. INTRODUCTION

The concept of biodiversity includes species richness and the diversity of habitats and ecosystems in which organisms occur (Neigel, 1999). The history and biogeographic location of an area, as well as interspecific interactions, can affect populational processes by influencing on the patterns of abundance, richness and spatial distribution of organisms in many communities (Connell, 1972; Paine, 1974; Menge & Sutherland, 1976; Karlson, 1980; Ricklefs, 1996; Robles & Desharnais, 2002).

Marine intertidal organisms are usually distributed in conspicuous zones or patches, as a result of limiting physical factors, such as space, or by the presence of predators or competitors that are confined to specific areas of the habitat (Paine, 1974; Jackson, 1977; Sebens, 1982). Physiological differences among sessile animals on intertidal rocky shores can lead to variations in resistance to desiccation, salinity and temperature fluctuations, that can influence the fixation of larvae and the survival of adults on the substratum (Lubchenco, 1980; Nybakken, 1993). Thus, the zonation of species is the main characteristic of intertidal communities (Paine, 1974; Jackson, 1977).

Ascidians (Subphylum Tunicata, Class Ascidiacea) are exclusively sessile marine invertebrates found throughout the world, and occur in shallow to deep water (Rocha, 1988; Rodrigues *et al.*, 1999). Most species are found attached to rocks, shells, seaweeds stems and ship hulls, but may also be fixed in mud or sand through filaments or by a stolon (Monniot *et al.*, 1991).

Ascidian zonation is determined mainly by differences between the species tolerance to wave action, exposure to air, and water turbidity, with the latter being directly related to the availability of oxygen and food (Berril, 1932). The influence of light on

ascidian larvae is well known, and is one of the main factors controlling the microdistribution of these animals (Millar, 1971). According to Dybern (1969), the choice of a recruitment site by the larvae determines the distribution pattern of the species, with the light intensity directly affecting microhabitat selection. Laubier (1966) *apud* Millar (1971) listed the microhabitats of 21 ascidian species, and found only three photophilic species. In addition, the directional growth capacity of colonial species allows the exploration of refuges that are unexpected during the larval period (Connell, 1961, 1970; Meadows & Campbell, 1972; Buss, 1979). This ability increases the chances that young colonies will survive and reproduce (Hunt & Scheibling, 1997; Maldonado & Uriz, 1998).

Lambert & Lambert (1998) observed a high rate of introduction of exotic species in harbors in California. Similar findings have been reported for the São Sebastião Channel, in the Brazilian State of São Paulo (Rodrigues & Rocha, 1993; Rocha & Monniot, 1995; Rodrigues *et al.*, 1999). According to Monniot *et al.* (1991), the emergence of exotic species in many harbors is a worldwide phenomenon and can be explained by the easy recruitment and fast growth of ascidians on ship hulls that have not been painted recently (Keough, 1984; Rodrigues *et al.*, 1999). Such dispersal by ships can extend the distribution range of species worldwide (Lambert & Lambert, 1998).

Dybern (1965) reported marked seasonality in the abundance of ascidian larvae in the plankton of temperate waters, mainly because these organisms are sensitive to low temperatures. Yamaguchi (1975) recorded similar findings, for colonial and solitary ascidians; with an increase in the time required for the animals to double its size as the temperature decreased. The greatest climatic homogeneity throughout the year in the tropics suggests that variations in ascidian abundance should be less pronounced. However, Rocha (1991) found marked yearly variation in the number of colonies along the coast

around São Sebastião, and suggested that this was related to the mean monthly temperatures. Other reports have also demonstrated the effects of temperature on community seasonality on the Brazilian coast (Oliveira Filho & Mayal, 1976; Rocha *et al.*, 1999).

In this work, we investigated the composition, richness and diversity of colonial ascidians in the intertidal zone of two rocky beaches, Praia Grande and Praia da Baleia, close to São Sebastião. Specifically, the distribution pattern, zonation, seasonality and microhabitat occupation of the species were examined.

### **1.3. MATERIAL AND METHODS**

#### **1.3.1. Study area**

The study was done at two sites close to and within the São Sebastião Channel. This channel, which is located on the northern coast of São Paulo State (23°43' S and 23°53' S, 45°20' W and 45°27' W) is 25 km long and 6-7 km wide at the two entrances, but only 2 km wide in the central region (Figure 1); the maximum depth is 40 m. Almeida (1964) *apud* Rocha (1988) considered the channel to be of erosive origin, having been dug during the last glacial stages of the Pleistocene.

Both the continental and island sides of the channel have small beaches delimited by prominent, extensive shores. These shores usually have a side that is more exposed to the movement of water and one that is sheltered (Giordano, 1986) with the latter often showing a small accumulation of rocks (Rocha, 1988).

The two sites were the rocky shores of Praia Grande ( $23^{\circ}49'23''$  S;  $45^{\circ}25'01''$  W), located inside the São Sebastião Channel and with a low exposure to wave action, and Praia da Baleia ( $23^{\circ}46'48''$  S;  $45^{\circ}39'50''$  W), located outside the channel and with greater exposure to wave action (Figure 1). Both beaches have an intertidal zone approximately 20 m wide that consists of rocks of different sizes. Whereas on Praia Grande the intertidal zone never exceeded 15 m, on Praia da Baleia, which sloped more gradually, the intertidal zone reached 40 meters in length. These two beaches also differed in their distribution of rocks: on Praia da Baleia, the rocky shore extended over the entire subtidal zone, while on Praia Grande, the rocky shore ended on the low intertidal zone, where the sandy bottom started.

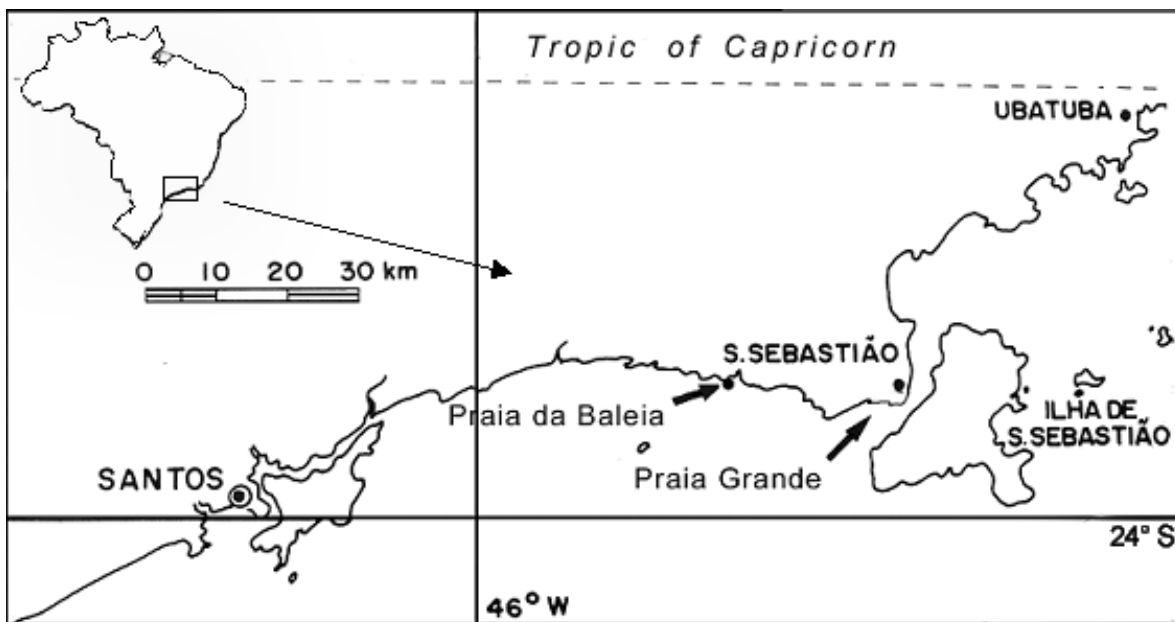


Figure 1. Study area in southeastern Brazil (modified from Rodrigues & Rocha, 1993).

### 1.3.2. Sample collection

Samples were collected monthly, from January to November 2001 on Praia da Baleia and from February 2002 to January 2003 on Praia Grande, always at low tide.

In the intertidal zone, three belt transects approximately 40 m long and 5 m wide were delimited for Praia da Baleia and two transects 15 m long and 10 m wide for Praia Grande. Each transect on Praia da Baleia was divided into three strata of 10 m long separated by 5 m while on Praia Grande the strata were adjacent and 5 m long. In all cases, the strata corresponded to the high, middle and low intertidal zone.

For sampling, a transect was randomly selected from each one of the strata in the rocky intertidal zone. In each stratum (high, middle and low intertidal), five 1m<sup>2</sup> quadrats were also randomly delimited (Brower & Zar, 1984). For each quadrat, the number of species present, the area covered by each species and the microhabitats they occupied were recorded. The microhabitats were defined as: rock undersurface, upper surface, lateral surface, abandoned sea-urchin hole, rock crevice, negative surface of rock and seaweed stem.

The most abundant and representative species on each beach (eight species at Praia da Baleia and 15 at Praia Grande) were used to assess the space distribution and microhabitat occupation.

When necessary, samples of each species were collected for subsequent identification. The specimens were anesthetized with menthol and preserved in 10% neutral formalin in seawater (Lotufo, 1997). The specimens were identified to the species level based on the descriptions of Rodrigues *et al.* (1998), Lotufo (2002) and Rocha & Monniot (1995) for the family Didemnidae. The identifications were confirmed and doubtful cases



were resolved with the help of Dr. Sérgio de Almeida Rodrigues and Dr. Tito Monteiro da Cruz Lotufo.

### 1.3.3. Statistical analysis

The statistical analyses involved comparisons among intertidal zone strata, seasons, beaches and microhabitats. The densities and the area covered by the ascidian species were compared using the Kruskal-Wallis test, a non-parametric test for variance analysis, followed by the Bonferroni or Dunn test (Brower & Zar, 1984; Zar, 1999). All comparisons were done using the software Bioestat 2.0 (Ayres *et al.*, 2000). All data for the area covered throughout the year by species that differed from normality were transformed by  $\log(x+1)$  to facilitate the comparisons.

The correlation between the mean area covered by each community and the mean monthly water temperature was assessed using the Spearman rank correlation test (Rocha, 1991). The water temperature data were obtained from the Centro de Biologia Marinha da USP (CEBIMar) at São Sebastião.

The Shannon-Wiener index was used to determine the diversity of the strata, communities and seasons as follows:

$$H' = -\sum_{i=1}^k p_i \ln p_i$$

where  $H'$  = Shannon-Wiener diversity index,  $p_i = n_i/N$ ,  $n_i$  = area covered by the  $i$ th species and  $N$  = total area covered.

The diversity indices were compared by the T test, corrected by the Bonferroni procedure for multiple comparisons (Brower & Zar, 1984).

The evenness of Pielou, which expresses the observed diversity as a proportion of the maximum possible diversity and provides important data about the relative species abundance was calculated (Zar, 1999) as:

$$J' = H'/H_{\text{MÁX}}$$

where  $H'$  = Shannon-Wiener diversity index,  $H_{\text{MÁX}} = \ln S$  and  $S$  = number of species.

Dominancy was calculated according to Brower & Zar (1984) as:

$$D = 1/J'$$

where  $D$  = dominancy and  $J'$  = Pielou evenness

The Sorensen index was used to compare similarities, using the relationship:

$$CC_s = 2C/(s_1 + s_2)$$

where  $CC_s$  = Sorensen index,  $C$  = number of species common to two samples or two communities,  $s_1$  = number of species in sample 1 or community 1 and  $s_2$  = number of species in sample 2 or community 2.

## 1.4. RESULTS

### 1.4.1. Species composition

Thirty-three species of colonial ascidians belonging to two orders (Enterogona and Pleurogona), three suborders (Aplousobranchia, Phlebobranchia and Stolidobranchia) and six families (Didemnidae, Clavelinidae, Holozoidae, Polyclinidae, Perophoridae and Styelidae) were recorded from Praia Grande and Praia da Baleia (Table 1).

The species are described in Apêndice and will form an illustrated field guide for non-specialists. *Didemnum* sp. n1 and *Didemnum* sp. n2, are two new species described in the second chapter of this dissertation.

The largest number of species (19) belonged to the family Didemnidae, followed by Styelidae (8). Four *Didemnum*, one *Trididemnum* and one *Aplidium* species were not identified to the species level because not all of the taxonomic characters required were found or because only a small amount of material was available (in the case of *Trididemnum*).

The Sorensen Index indicated 58% similarity in the species composition between the two beaches. The two sampled beaches showed similar relative species compositions among the three intertidal strata. The largest similarity was found between the low and middle strata (79% and 94% for Praia da Baleia and Praia Grande, respectively) followed by the middle and high strata, (75% and 89%, respectively) and by the low and high strata (50% and 81%, respectively). In all cases Praia Grande showed the largest similarity among strata.

Table 1. Species sampled during this study and their place of occurrence.

Sub-Order	Family	Species	P. Baleia	P. Grande
Aplousobranchia	Didemnidae	<i>Didemnum cineraceum</i>		•
		<i>Didemnum granulatum</i>	•	•
		<i>Didemnum ligulum</i>		•
		<i>Didemnum lutarium</i>	•	•
		<i>Didemnum perlucidum</i>		•
		<i>Didemnum psammatodes</i>		•
		<i>Didemnum speciosum</i>		•
		<i>Didemnum vanderhorsti</i>	•	•
		<i>Didemnum apersum</i>		•
		<i>Didemnum</i> sp. n1	•	
		<i>Didemnum</i> sp. n2		•
		<i>Didemnum</i> sp.1	•	•
		<i>Didemnum</i> sp.2	•	
		<i>Didemnum</i> sp.3	•	
		<i>Didemnum</i> sp.4		•
		<i>Diplosoma macdonaldi</i>	•	•
		<i>Trididemnum orbiculatum</i>	•	•
		<i>Trididemnum</i> sp.	•	
		<i>Polysyncraton amethysteum</i>	•	•
			Clavelinidae	<i>Clavelina oblonga</i>
	Holozoidae	<i>Distaplia bermudensis</i>		•
	Polyclinidae	<i>Aplidium</i> sp.		•
		<i>Polyclinum constellatum</i>	•	•
Phlebobranchia	Perophoridae	<i>Perophora multiclathrata</i>	•	
		<i>Perophora viridis</i>		•
Stolidobranchia	Styelidae	<i>Botrylloides giganteum</i>	•	•
		<i>Botrylloides nigrum</i>		•
		<i>Botryllus tabori</i>	•	•
		<i>Botryllus tuberatus</i>	•	•
		<i>Eusynstyela tinctoria</i>	•	•
		<i>Polyandrocarpa zorritensis</i>	•	
		<i>Symplegma rubra</i>	•	•
		<i>Symplegma brakenhielmi</i>	•	•

### 1.4.2. Ascidian distribution along the intertidal zone

The area covered by the taxocoene of colonial ascidians along the intertidal zone differed between the two beaches. At Praia da Baleia, there was a preference for the low intertidal stratum ( $p < 0.0001$ ), while at Praia Grande the middle stratum was the most used ( $p = 0.014$ ). On both beaches, the two remaining strata were equally occupied (Figure 2). The total area covered by ascidians at Praia Grande was larger than at Praia da Baleia, a pattern also seen in the middle and high strata ( $p < 0.0001$ ). The low stratum showed similar level of occupation on both beaches ( $p = 0.30$ ).

Of the eight most abundant species at Praia da Baleia, five (*Didemnum* sp. n1, *Didemnum* sp.1, *Didemnum* sp.2, *Diplosoma macdonaldi*, and *Polysyncraton amethysteum*) preferentially occupied the low intertidal stratum, in agreement with the pattern observed for the total taxocoene. Two (*Trididemnum orbiculatum* and *Didemnum granulatum*) occupied the low and middle strata equally, and were rarely found in the upper stratum, whereas *Symplegma brakenhielmi* occupied the entire intertidal zone equally (Figure 3).

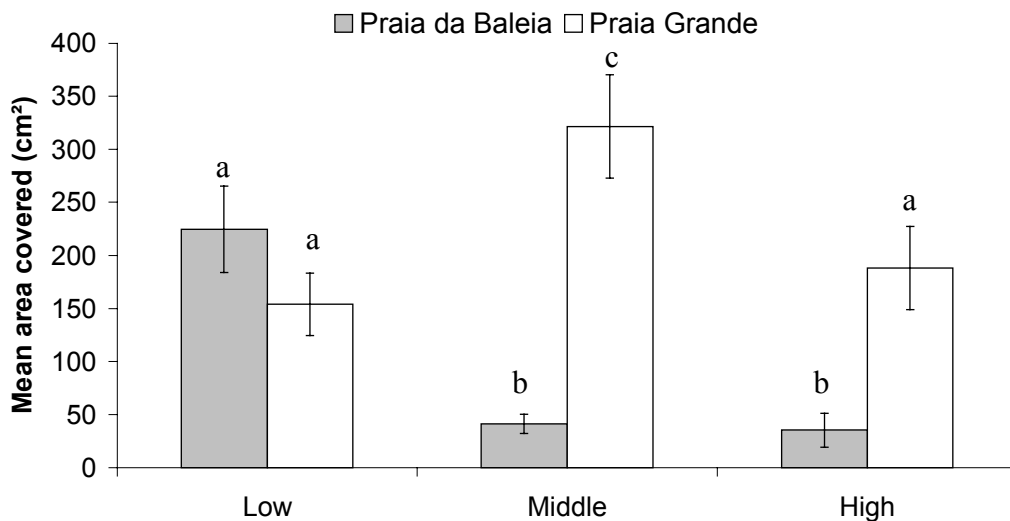


Figure 2. Area covered by ascidians per quadrat in each stratum on the two beaches studied. The columns are the mean  $\pm$  standard error of area covered.

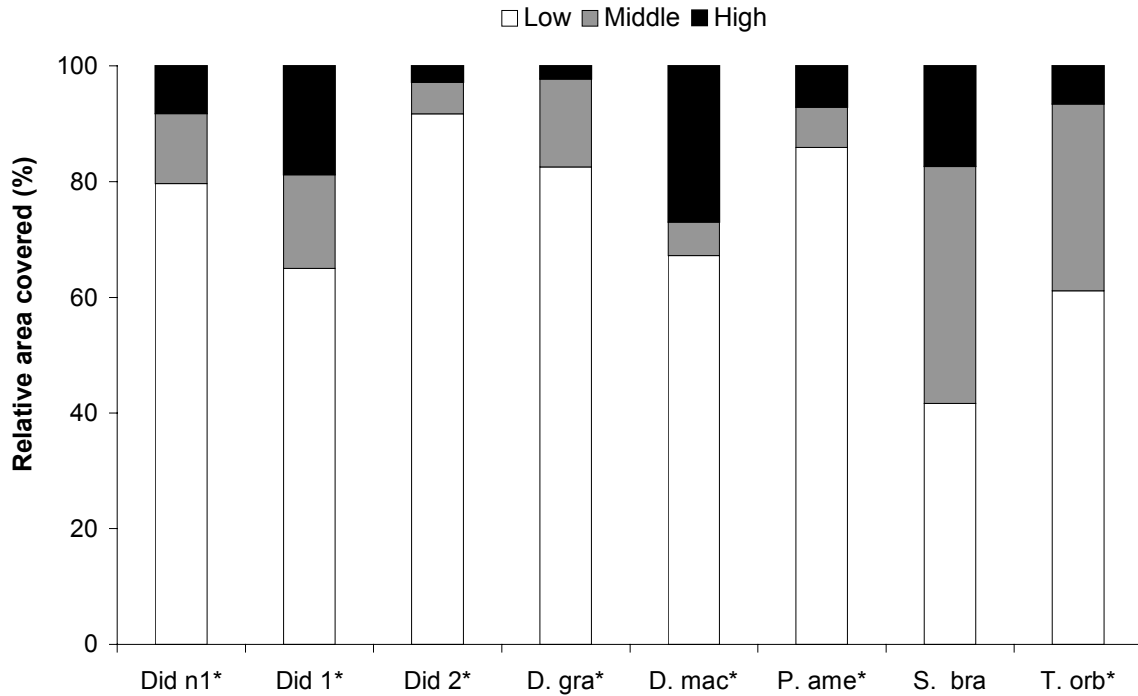


Figure 3. Relative area covered in the three strata by the eight most abundant species at Praia da Baleia. \* =  $p < 0.05$ . Did n1 = *Didemnum* sp. n1; Did 1 = *Didemnum* sp.1; Did 2 = *Didemnum* sp.2; D.gra = *Didemnum granulatum*; D. mac = *Diplosoma macdonaldi*; P.ame = *Polysyncraton amethystrum*; S.bra = *Symplegma brakenhielmi*; T.orb = *Trididemnum orbiculatum*.

Of the 15 most abundant species at Praia Grande, six (*Botrylloides nigrum*, *Botryllus tuberatus*, *Diplosoma macdonaldi*, *Didemnum vanderhorsti*, *Didemnum* sp.1 and *Polysyncraton amethystrum*) occupied all the strata of the intertidal zone to equal extents, four (*Aplidium* sp., *Botrylloides giganteum*, *Symplegma rubra* and *Didemnum granulatum*) preferentially occupied the middle stratum, three (*Trididemnum orbiculatum*, *Botryllus tabori* and *Didemnum* sp.4) were more abundant in the low and middle strata, and were rarely found in the high intertidal, and the remaining two species (*Symplegma brakenhielmi* and *Didemnum psammathodes*) showed an inverted pattern, being abundant in the middle and high strata (Figure 4). The species distribution in this area was thus different from the pattern seen at Praia da Baleia.

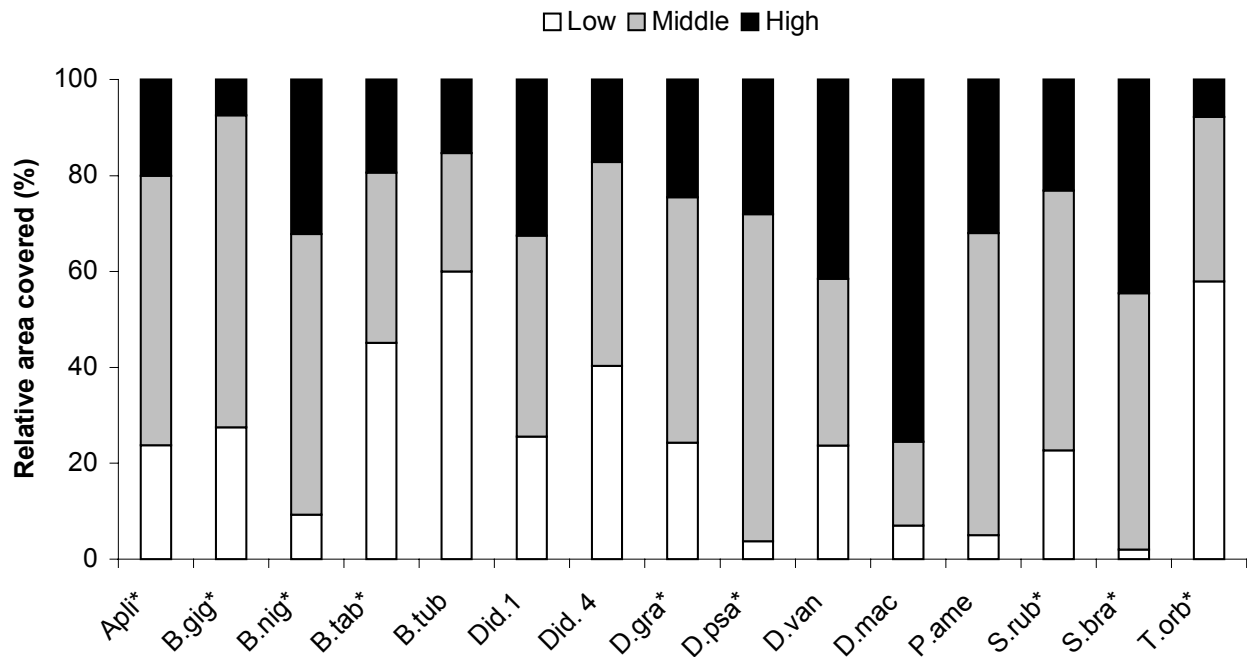


Figure 4. Relative area covered in the three strata by the 15 most abundant species at Praia Grande. \* =  $p < 0.05$ ; Apli = *Aplidium* sp.; B.gig = *Botrylloides giganteum*; B.nig = *Botrylloides nigrum*; B.tab = *Botryllus tabori*; B.tub = *Botryllus tuberatus*; Did.1 = *Didemnum* sp.1; Did.4 = *Didemnum* sp.4; D.gra = *Didemnum granulatum*; D.psa = *Didemnum psammatoedes*; D.van = *Didemnum vanderhorsti*; D.mac = *Diplosoma macdonaldi*; P.ame = *Polysyncrator amethysteum*; S.rub = *Symplegma rubra*; S.bra = *Symplegma brakenhielmi*; T.orb = *Trididemnum orbiculatum*.

Among the six species common to both beaches, only *T. orbiculatum* showed a similar distribution on both shores. *Diplosoma macdonaldi* tended to occupy the highest stratum at the two sites but because of its seasonality (see below) was found in insufficient number of quadrats to detect significance. The remaining species were more widely distributed on Praia Grande.

The species richness showed a similar pattern to that of the area covered, i.e. usually greater at Praia Grande, with the lowest stratum not differing between beaches ( $p > 0.05$ ). The richest area at Praia da Baleia was the lowest intertidal stratum ( $p < 0.0001$ ), while at Praia Grande the middle stratum presented the greater number of species per quadrat ( $p < 0.001$ ). On both beaches, the two remaining strata were equally rich (Figure 5).

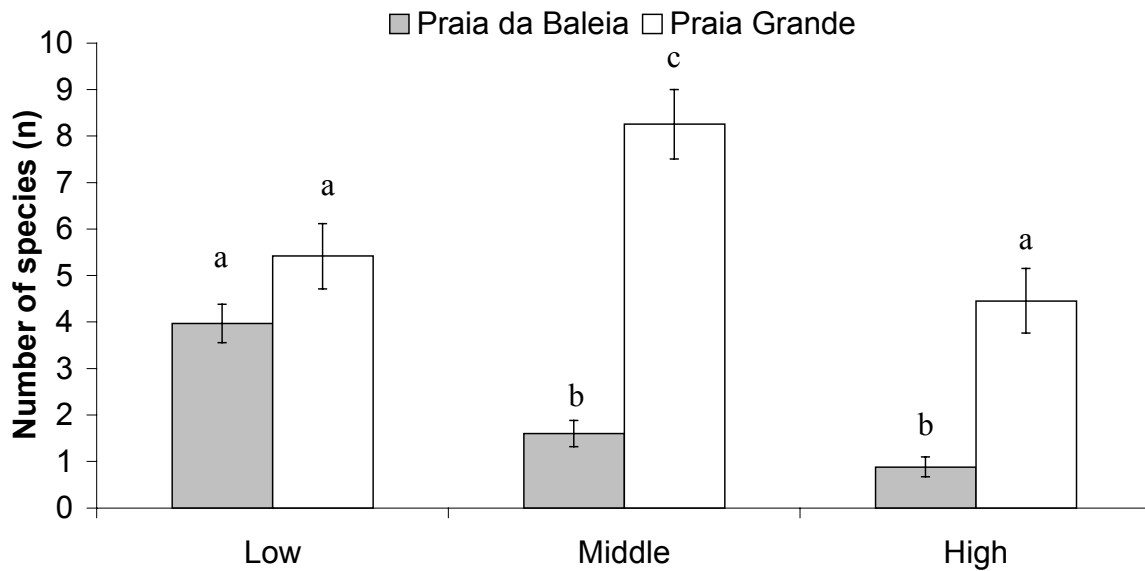


Figure 5. Species richness per quadrat in the three strata of the two beaches studied. The columns are the mean  $\pm$  standard error of number of species.

The species diversity on Praia Grande was greater comparing both, the total area ( $p=0.003$ ), and for each of the three strata separately ( $p_{\text{low}}=0.018$ ;  $p_{\text{middle}}=0.023$ ;  $p_{\text{high}}=0.0001$ ). Comparisons among the three strata for each beach showed that the only difference was between the middle and high intertidal strata on Praia da Baleia, with the high stratum showing the smallest diversity ( $p<0.05$ ). There were no differences in the diversity indices among the three intertidal strata at Praia Grande (Figure 6).

The evenness at Praia Grande was similar in the three intertidal zone strata and resulted in equivalent dominance ( $D_{\text{Low}}=1.37$ ,  $D_{\text{Mid}}=1.39$ ,  $D_{\text{High}}=1.32$ ). In contrast, at Praia da Baleia there were significant differences among the evenness values, with the highest dominance in the high intertidal zone ( $D_{\text{Low}}=1.39$ ,  $D_{\text{Mid}}=1.20$ ,  $D_{\text{High}}=1.54$ ) (Figure 7).



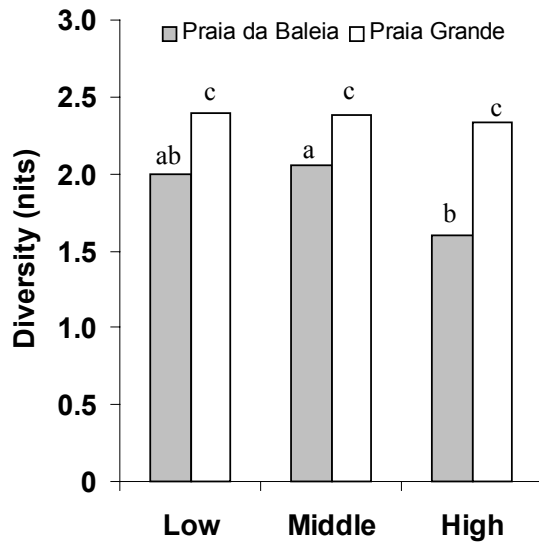


Figure 6. Species diversity in the three strata of the two beaches studied.

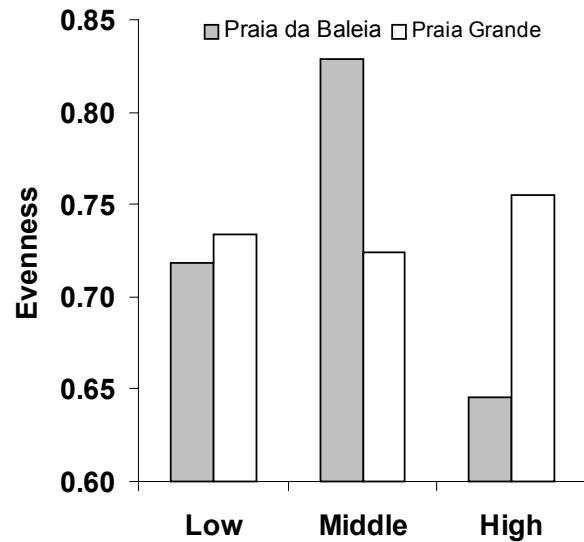


Figure 7. Evenness in the three strata of the two beaches studied.

### 1.4.3. Community seasonality

The mean area covered throughout the year was correlated with the monthly average temperature when the data of both beaches were analyzed together ( $p=0.012$ ). When analyzed individually, there was no significant correlation for either beach (Figure 8 and 9).

The two beaches differed in their taxocoene abundance during the year. At Praia da Baleia, the extent of coverage increased from the beginning of spring (end of August), onwards and then decreased at the beginning of autumn (Figure 8). At Praia Grande, there was an increase in the area covered in August, and another increase at the beginning of summer (December) (Figure 9), similar to the pattern observed at Praia da Baleia. An additional peak in March coincided with the beginning of the decrease seen at Praia da Baleia.

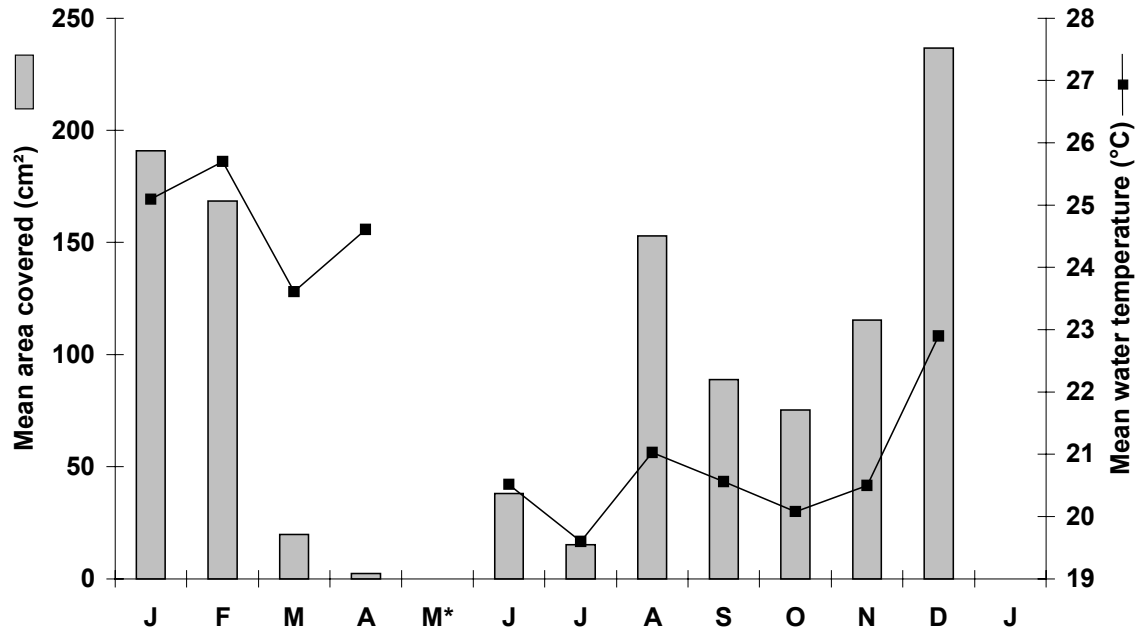


Figure 8. Mean area coverage per quadrat at Praia da Baleia and mean monthly water temperature during 2001. \* No data collected because of adverse climatic conditions.

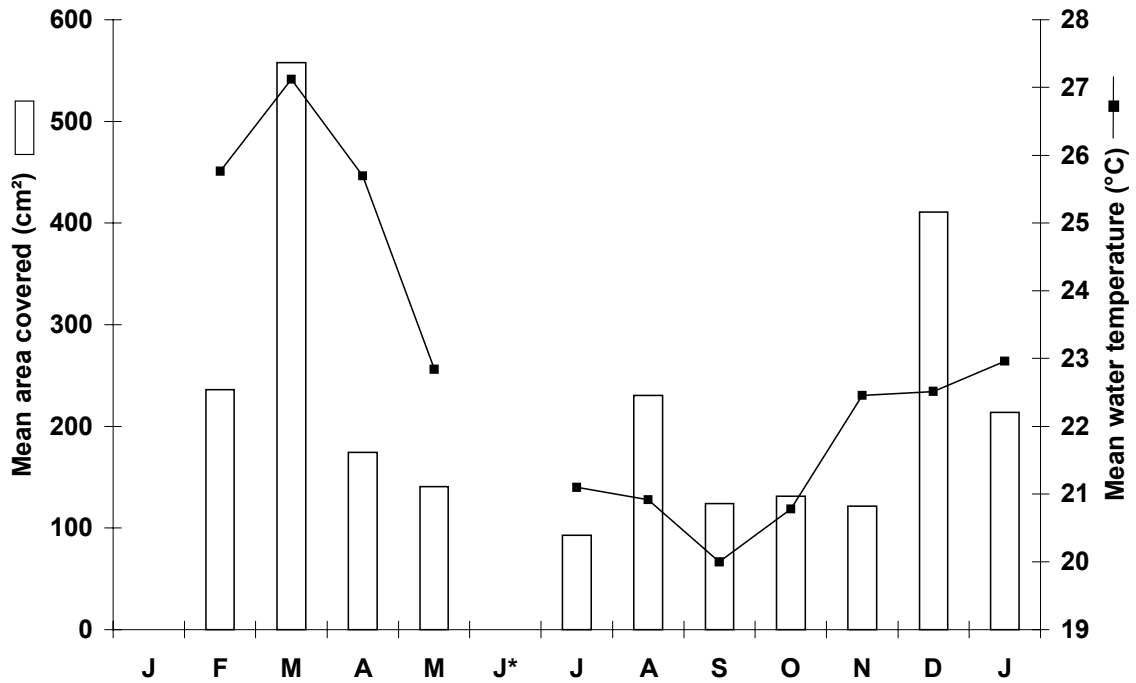


Figure 9. Mean area coverage per quadrat at Praia Grande and mean monthly water temperature during 2002/3. \* No data collected because of adverse climatic conditions.

Two species showed patterns of abundance during the year that differed from the general community pattern. On Praia Grande, *Didemnum granulatum* peaked at the beginning of autumn and spring, and was quite abundant throughout the year (Figure 10A), whereas on Praia da Baleia this species showed the same pattern of abundance seen for the whole taxocoene. A second species, *Diplosoma macdonaldi*, which was present on both beaches showed a single peak in the year, followed by reduced coverage in subsequent months (Figure 10B).

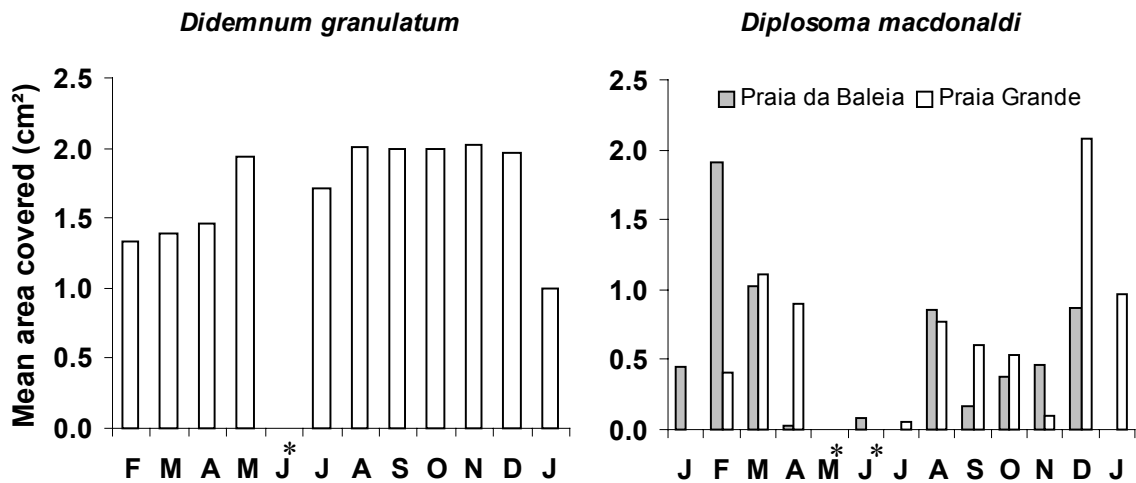


Figure 10. Mean area covered per quadrat (log x+1) by A) *Didemnum granulatum* on Praia Grande and B) *Diplosoma macdonaldi* on both beaches. \* No data obtained because of adverse climatic conditions, in May (Praia da Baleia) and in June (Praia Grande).

The species richness at Praia da Baleia and Praia Grande initially stabilized, with a few quadrat sampled, at 16 and 24 species, respectively (Figure 11). This number were maintained until the end of winter and beginning of spring, when a new increase in species richness was seen (4 species per beach). This increase agreed with the increase in area covered and indicated invasion by previously absent species.

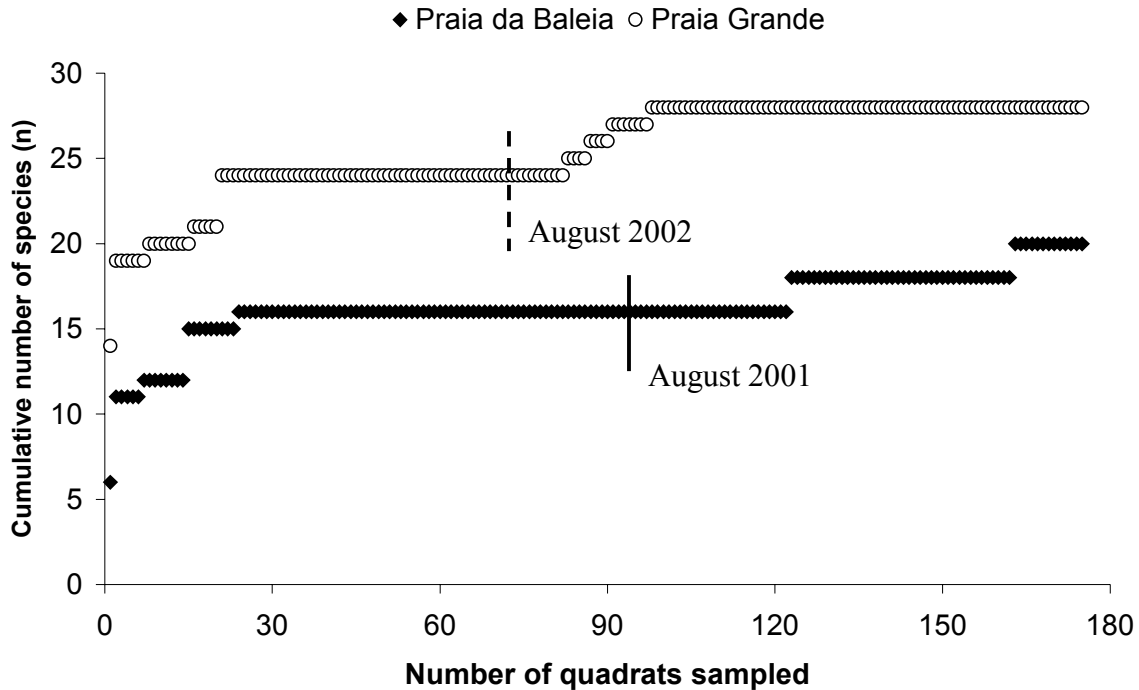


Figure 11. Cumulative number of species per number of sampled quadrats for both beaches.

The mean species richness per quadrat was greater on both beaches in the summer. However, on Praia da Baleia, the richness in the summer months was similar to that in the spring and winter with these being higher than in the autumn ( $p=0.001$ ). On Praia Grande, the species richness in the summer did not differ from that seen in the autumn, being greater than in the winter and spring ( $p<0.001$ ) (Figure 12).

The diversity throughout the year showed a similar pattern to the mean species richness on both beaches (Figure 13). Differences in the evenness on Praia da Baleia indicated a greater dominance in the summer and a smaller value in the winter (Figure 14). On Praia Grande the patterns for richness and diversity were also maintained in the evenness, with the summer months being simultaneously the richest, most diversified, and with a more equal distribution of coverage. The smallest values for these three attributes were observed, in general, in the autumn at Praia da Baleia and spring at Praia Grande.

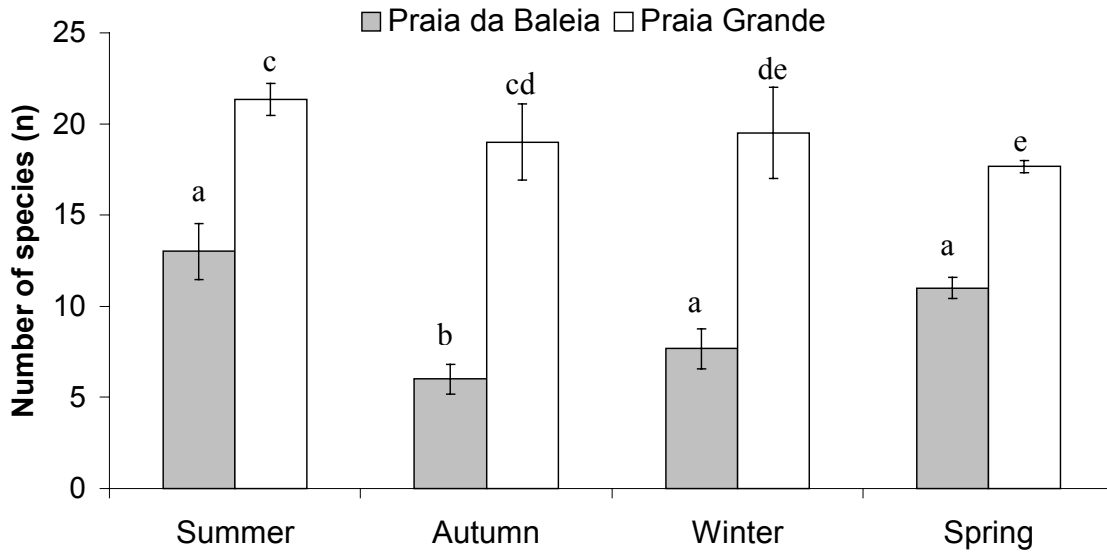


Figure 12. The mean seasonal species richness per quadrat for the two beaches studied. The columns are the mean  $\pm$  standard error of number of species.

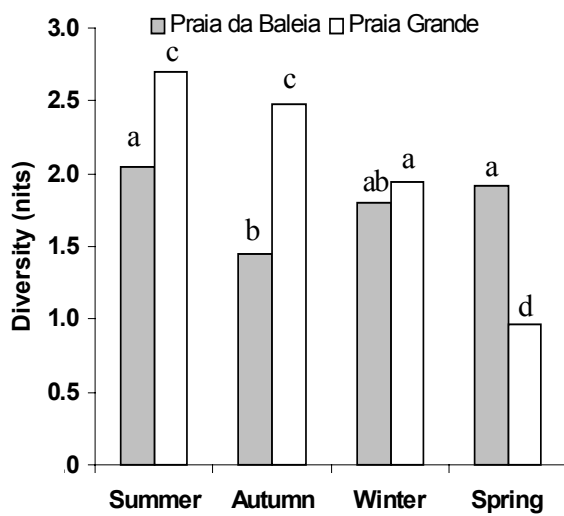


Figure 13. Seasonal species diversity for the two beaches studied.

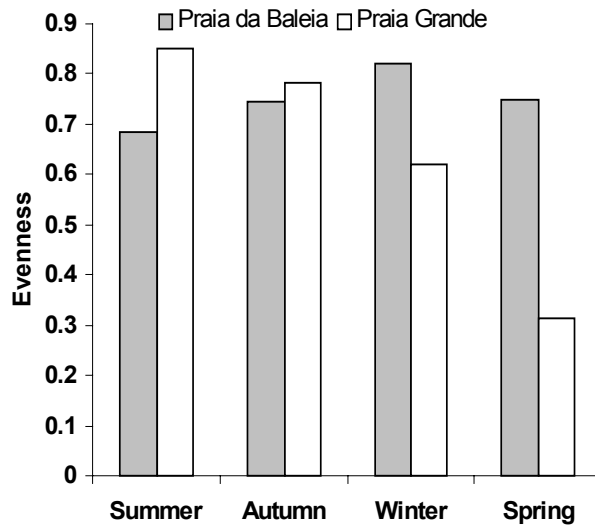


Figure 14. Evenness for the two beaches studied.

#### 1.4.4. Microhabitat occupation

Most ascidians were found on the undersurface of rocks. The six other microhabitats examined were occupied to different extents on the two beaches. On Praia da Baleia, the second most occupied microhabitat (lateral surface of rocks) accounted for <6% of the total area occupied by the ascidians compared to 84% of the undersurface and there were no colonies on the upper surface of rocks (Figure 15). This pattern of occupation was common to six of the eight species for which the microhabitats were analyzed (*Didemnum granulatum*, *Didemnum* sp.1, *Didemnum* sp.2, *Didemnum* sp. n1, *Diplosoma macdonaldi* and *Trididemnum orbiculatum*). The two remaining species, *Polysyncraton amethysteum* and *Symplegma brakenhielmi* showed no specific patterns of occupation.

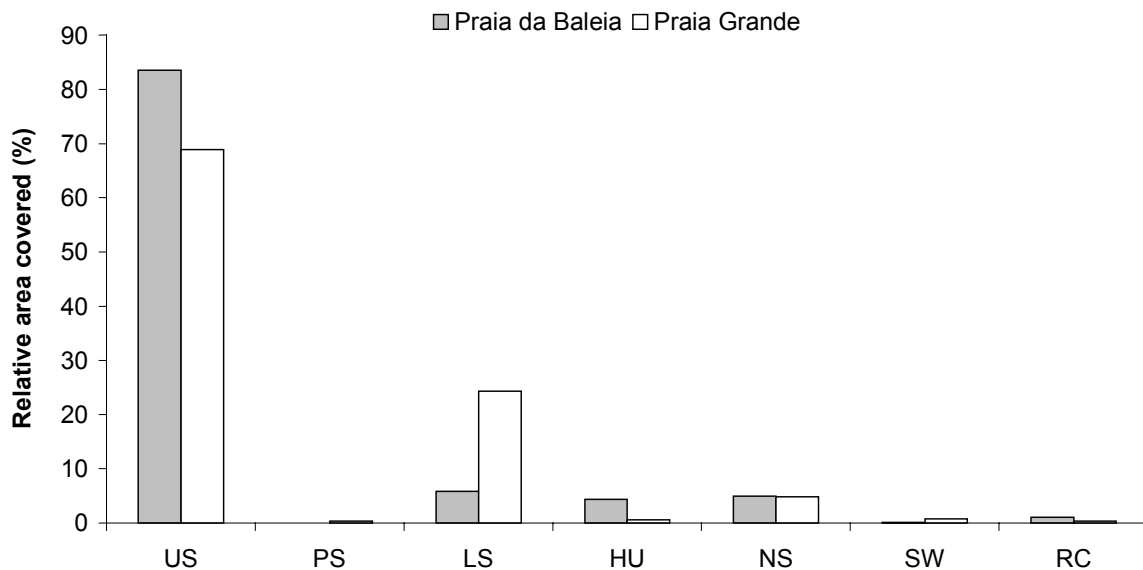


Figure 15. Relative area covered by ascidians in seven microhabitats on the two beaches studied. US = undersurface of rock; PS = upper surface of rock; LS = lateral surface of rock; HU = abandoned sea-urchin hole; NS = negative surface of rock; SW = seaweed stem; RC = rock crevice.

The undersurface of rocks accounted for 69% of the total area occupied by ascidians on Praia Grande compared to 24% for the lateral surface. Thus there was a greater use of

this microhabitat than on Praia da Baleia. In addition, a few colonies were recorded on the upper surface of rocks on Praia Grande (Figure 15).

Eleven of the 15 species whose microhabitats were analyzed on Praia Grade showed more covering on the lateral and undersurface of rocks, when compared to the remaining microhabitats. Three species (*Botryllus tuberatus*, *Botrylloides nigrum* and *Polysyncraton amethysteum*) occupied all of the microhabitats equally and one (*Symplegma brakenhielmi*) preferentially occupied the undersurface of rocks.

Among the 11 species that preferentially occupied the undersurface and lateral surfaces of rocks, five (*Botryllus tabori*, *Didemnum granulatum*, *Didemnum psammatodes*, *Didemnum vanderhorsti* and *Didemnum* sp.1) covered a greater area on the undersurface than on the lateral surface. The six remaining (*Aplidium* sp., *Botrylloides giganteum*, *Didemnum* sp.4, *Diplosoma macdonaldi* and *Trididemnum orbiculatum*) did not differ in their occupation of these two microhabitats.

In contrast to all of the other species, *Didemnum speciosum*, which was recorded only a few times during the study, was always found growing on stems of seaweed, and in no other microhabitat.

## 1.5. DISCUSSION

The ascidian species richness in the two areas studied here was relatively high when compared with other reports (Rocha, 1988; Lotufo, 1997), particularly since only the intertidal zone was investigated. In addition of the 33 species recorded, two were new

species. Rocha (1988) registered 13 species of colonial ascidians at the same area, while Lotufo (1997) recorded 21 species at Baía de Santos.

The predominance of the family Didemnidae observed is characteristic of tropical and subtropical waters. However, the reduced size of the zooids, the small number of taxonomic characters (Monniot *et al.*, 1991), and the still poor knowledge of the species of this and others families found on the Brazilian coast, hampered the identification of three *Didemnum*, one *Trididemnum* and one *Aplidium* morphotypes.

The quantification of the area covered by ascidians proved to be efficient for assessing the composition and abundance of these sessile animals. This approach was initially suggested by Millar (1971), but there have been no reports describing its large-scale application to ascidian colonies. An exception to this is the work of Lotufo (1997) that analyzed on reproductive and successional aspects of ascidians.

The difference in species composition between the two beaches probably reflected the influence factors such as wave impact, which was greater at Praia da Baleia. Wave impact could reduce the recruitment of less resistant species and could explain the lowest species richness of this beach (Raffaelli & Hawkins, 1996). On the other hand, the greater richness found at Praia Grande may be a result of the intense movement of ships through the São Sebastião Channel. Many of these vessels transport species of colonial ascidians that could release larvae into the channel (Lambert & Lambert, 1998). In addition, compared to Praia da Baleia, Praia Grande is a sheltered rocky shore, and can harbor more fragile species.

Comparison of the species sampled at Praia Grande in this work with those reported by Rocha (1993) for the same site ten years earlier indicated an increase in richness. This increase may reflect introduction of exotic species. No *Lissoclinum fragile*, *Eudistoma* sp.



and *Didemnum rodriguesi* were found among the colonial ascidians sampled here. These species apparently no longer occur in the intertidal zone of Praia Grande, thus suggesting important modification in the ascidian taxocoene of this region. Rocha & Monniot (1993) reported that *Didemnum rodriguesi* was abundant in the area studied here.

In contrast, seven species not recorded by Rocha (1993) were found on this beach; including *Symplegma rubra*, *Polysyncraton amethysteum*, *Didemnum apersum*, *Didemnum cineraceum*, *Didemnum ligulum*, *Didemnum psammathodes* and *Perophora viridis*. This finding indicates that there was an overall increase in the local species richness during the previous 10 years, particularly in Didemnidae, or more probably, this fact may reflect the different methods of analysis used, as well as recent changes to the taxonomy of this group. Although Lambert & Lambert (1998) also reported an elevated rate of introduction for didemnid species close to harbors, related with an intensive movement of ships.

There was a greater similarity between the faunas of the lower and middle strata at both beaches, probably because of the smaller variation in environmental factors in these regions. The upper stratum was more affected by variations in physical factors, such as a longer exposure to air during low tides, because of its distance from the average water line. Thus, only the more resistant species are expected to be found there (Meadows & Campbell, 1972; Buss, 1979; Hunt & Scheibling, 1997). Another explanation for the lower richness of this stratum is the smaller recruitment in this area of the shore, because of the higher larvae mortality and difficulties to access it (Underwood & Denley, 1984).

The findings that Praia Grande had a higher species similarity among the strata than did Praia da Baleia was probably associated with the smaller extent of its intertidal zone and larger area covered by colonial ascidians. These factors could increase the probability of a species being sampled in the three strata, even when present in very different

proportions (Little & Kitching, 1996). In this case, the Sorensen index does not consider the area of the colonies. According to Sebens (1991), an increase in environment fragmentation, as seen at Praia da Baleia, generally makes the assemblages more heterogeneous.

The distribution of colonial ascidians throughout the intertidal zone was probably influenced by different factors at the two beaches. Although the diversity and evenness did not differ among the three strata at Praia Grande, the species richness and area covered in the middle stratum were greater because of the impact of waves and exposure to air at the upper limit of this stratum (Menge & Sutherland, 1976) and larger sedimentation rates in the subtidal region may also have had an effect (Todd & Turner, 1986; Hurlbut, 1993; Daly & Mathielson, 1977). At Praia da Baleia, the greater extension of the rocky shore down to the subtidal reduced the effect of the sandy bottom in the intertidal zone, and allowed a greater occupation of the lower stratum, and, hence, greater richness and diversity closer to the subtidal zone.

The possibility that abiotic factors may control the lower limit of species distribution in the intertidal zone does not agree with theories established for temperate climate regions by Paine (1966) and Dayton (1971, 1975). However, various studies in tropical areas have shown such control. Studies such as those of Daly & Mathielson, (1977), Underwood (1980), Eckman (1983) and MacGuinness (1987) have found an elevated mortality or reduced diversity after the invasion by sand on rocky shores. Sessile colonial animals are generally the most affected by the abrasion resulting from sediment accumulation. Rocha (1993) observed sand invasion on the Praia Grande shore. Apart from influencing spatial distribution on the shore, this invasion by sand can also be one of the main factors controlling microhabitat occupation by colonial ascidians. In general, these

tunicates occupy mainly the undersurface of rocks, where the accumulation of sediments is smaller, thus reducing the effect of abrasion on the colony (Todd & Turner, 1986).

Other factors such as the photophobic behavior of the larvae and the degree of protection against predators and wave impact can also influence microhabitats selection (Millar, 1971). The sheltered shore at Praia Grande allowed the occupation of lateral rock surfaces. As a result, there was an increase in local richness since species that preferentially occupied this microhabitat (*Aplidium* sp. and *Didemnum* sp.4) were usually absent at Praia da Baleia. In addition, Praia Grande is frequently visited by tourists who have access to the shore at low tides (Rocha, 1988). The removal of predators, such as large gastropods and crabs by humans may also influence ascidians population growth and composition.

The lack of ascidian zonation at Praia da Baleia indicated that competition for space was not the principal factor controlling the distribution of species, what is corroborated by the large areas of bare rock seen on this beach.

The distribution of some species, such as *Symplegma brakenhielmi* and *Didemnum psammatodes*, in the upper stratum in Praia Grande suggested that these species were more resistant to environmental variations. At Praia da Baleia, *Symplegma brakenhielmi* was the only species to occur in this zone. *Didemnum psammatodes* shows an accumulation of fecal pellets in its tunic that could help to retain water during low tides. These two species were more abundant during the summer, when the lowest tides are nocturnal, thus minimizing the water loss by the colonies.

Most of the remaining species on Praia Grande occupied the middle stratum, although the presence of three species that preferred the lower and middle mediolitoral zones, indicated a more structured distribution on this shore. This structuring, which differed from that at Praia da Baleia, probably reflected competition for space in Praia

Grande, which showed an extensive area covered by ascidians and almost all of the species present on both beaches showed a more restricted distribution on the first one.

Although the species described here were sampled in different years, the abundance of the ascidians was always greater on Praia Grande than on Praia da Baleia. The summer months showed the largest areas of coverage, richness and diversity. The larger evenness on Praia Grande in this period indicated that, in addition to an increase in species richness, the dominance in the warmer months was minimal, i.e. species that were less abundant in the cold season showed an increase in area in the summer. In contrast, on Praia da Baleia, the larger diversity in the summer months was accompanied by a smaller evenness, indicating an increase in dominance, possibly caused by the disproportional growth of *Polysyncraton amethysteum* and *Didemnum* sp. n1, relative to the other species.

Millar (1971) and Rocha (1988) demonstrated the influence of the temperature on the number of larvae and colonies, respectively. The present study is one of the few to corroborate the importance of this abiotic factor in ascidian communities of tropical and subtropical regions, through quantitative data for the area of the colonies. Although, as discussed by Lotufo (1997), other factors related to seawater temperature can also affect the abundance of ascidians during the year. These factors are the most probable cause of the lack of correlation between the area covered and water temperature, when analyses are done using a small number of data (for each beach). Thus, for example, the beginning of storm-waves and the increased sedimentation seen on Praia da Baleia in April could be important factors in reducing the area covered by ascidians at this site (pers. obs.).

Some species of colonial ascidians did not follow the general pattern of abundance seen for the taxocoene throughout the year. Thus, *Diplosoma macdonaldi*, which was present on both beaches, occurred during a restricted period in the summer, disappearing or

showing a marked reduction in area throughout the rest of the year. Rocha (1991) studied the abundance of this species recruited on artificial substrata and found a less discrete peak of abundance. This same author noted that this species reached sexual maturity in less than one month. These observations, together with the very fragile tunic, suggest a low investment in body structures by *Diplosoma macdonaldi*, and may be indicative of an r strategist species among colonial ascidians.

In contrast to the other species, *Didemnum granulatum* was more abundant on Praia Grande at the end of winter and in the spring, and decreased at the beginning of summer. However, the area covered by this species on Praia da Baleia increased in the summer, indicating divergent behavior at the two beaches. A high resistance to low temperatures and a low ability to competition could explain the abundance of *Didemnum granulatum* during the year. However, a larger sample is required to confirm this hypothesis.

The increase of the covering area of ascidians in August, after its reduction on the coldest months is a combination of the enlargement of pre-settled colonies, the inward of pre-existent and as observed the recruitment of non-present species from the surrounding area. In conclusion, the results of this study showed that the ascidian communities were more dynamic than expected, and that difficulties associated with the short dispersal range of the ascidian larvae did not restrict the movement of the species between shores. Such movement could explain why species recorded by Rocha (1993) were not longer found on Praia Grande.

## **1.6. ACKNOWLEDGMENTS**

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## Capítulo 2

**Duas novas espécies para o gênero *Didemnum* no litoral sudeste  
do Brasil**





## 2.1. INTRODUCTION

Among ascidians, the family Didemnidae is, probably, the most common at intertidal and subtidal locations, especially in tropical regions where these species frequently form flat, encrusting colonies of different sizes, colors and thickness. As one of the most important components of the filter feeding and fouling communities, Didemnidae species show high growth rates that ensure they occupy most of the available substrate (Kott, 2001).

Didemnid zooids are the smallest of all colonial ascidians, and are generally only a few millimeters long. These zooids consist of thorax and abdomen and, because of simplification (Kott, 2001) have relatively few reliable morphological characters and overall uniformity. Larval characters and spicule form and size are other important taxonomic characters in this family. This combination of small size and few taxonomic relevant structures makes the identification of a species difficult, even for a specialist (Monniot *et al.*, 1991).

Along the Brazilian coast, the Didemnidae is represented by five of the eight known genera (Rodrigues & Rocha, 1993). These are distinguished by the absence or presence of spicules, the number of rows of stigmata (3 or 4), the number of testicular lobes (1, 2 or more), the format of the vas deferens (coiled or straight) and the absence or presence of an atrial siphon (Kott, 2001). The genus *Didemnum* Savigny, 1816 is the most diversified, with numerous species that are differentiated by a single or only a few morphological characters (Rocha & Monniot, 1995; Lotufo, 2002). In southeastern Brazil, species of this genus have been described by Rocha (1991), Rocha & Monniot, (1993) and Rodrigues & Rocha (1993).

The following papers describe two new species of *Didemnum* from the intertidal zone at São Sebastião, on the coast of the State of São Paulo, southeastern Brazil. The first species was collected on Praia da Baleia and is differentiated by its unique spicule shape. The second was collected on both Praia Grande and Praia Preta, and had a unique external appearance and divided testis.

## 2.2. *DIDEMNUM* sp. n1

RUNNING HEAD: A new species of *Didemnum*

TITLE: *Didemnum* sp. n1 nov., a new *Didemnum* (Tunicata: Ascidiacea) species from southeastern Brazil.

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### 2.2.1. ABSTRACT

A new species of *Didemnum* was discovered during a recent biodiversity survey of Praia da Baleia, São Sebastião. This species has a unique and peculiar type of spicule composed by four calcareous spheroids grouped together to form a tetrahedral structure. In other aspects, the new species is similar to certain other whitish tropical Atlantic American didemnids formerly assigned to *Didemnum candidum*.

### 2.2.2. INTRODUCTION

This paper describes a new species of *Didemnum* found at Praia da Baleia, São Sebastião, in the State of São Paulo, southeastern Brazil (23°46'48" S; 45°39'50" W), during a study biodiversity of intertidal compound ascidians. The specimens were collected from under boulders exposed at low tide. Compound ascidians from this region have been described by Rocha (1991), Rocha & Monniot (1993) and Rodrigues & Rocha (1993). The latter paper also lists older references on Brazilian ascidians and provides a map of the area.

Vouchers specimens of the examined material will be deposited in the Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP).

### 2.2.3. DESCRIPTION

*Didemnum* sp. n1 new species

(Figures 1-6)

Locality – Brazil, São Paulo Estate, Praia da Baleia, collected by Gustavo Muniz Dias.

Holotype – One colony in the MZUSP collected on 12/01/2003.

Paratypes – Two colonies in the MZUSP collected on 25/06/2003.

Diagnosis – Colonies thin, encrusting, whitish, and densely packed, with spicules of tetrahedral shape. Zooids less than 1 mm long, with testis undivided and spermiduct with 7-9 coils; ovary with one large egg, never simultaneously with the testis. Larvae with three papillae and four pairs of ampullae.

Etymology – The specific name refers to the peculiar aspect of the spicules (Fig. 1).

External appearance - Colonies very thin, less than 1 mm thick, sheet-like, less than 10 cm across, usually formed by small irregular plates anastomosing with one another on the upper surface. Color milky white, sometimes slightly orange (Fig. 2). Spicules of moderate size, around 30 µm across, densely packed throughout the test, tetrahedral in shape, formed by four rounded calcareous masses.

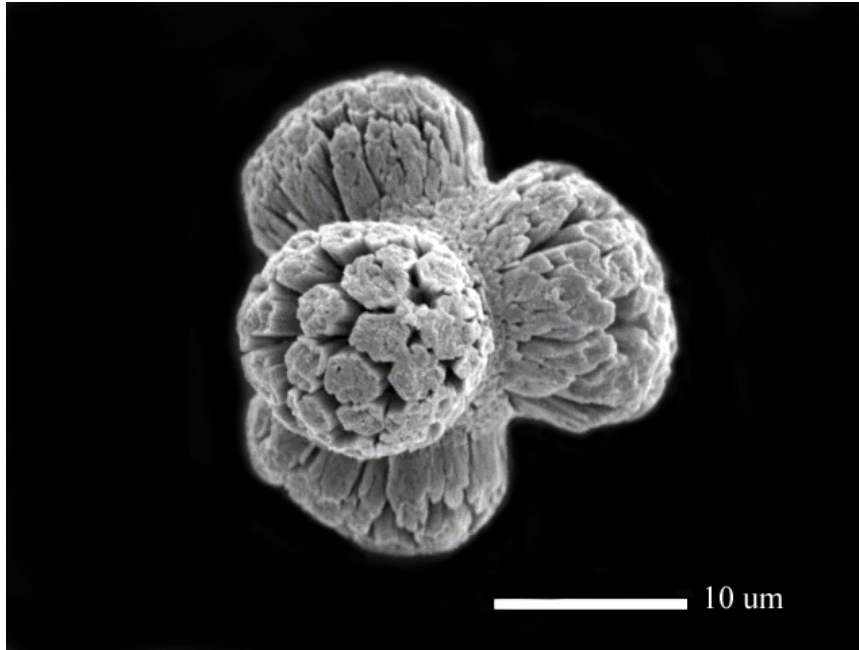


Figure 1. Electron microscopy of *Didemnum* sp. n1 spicule.

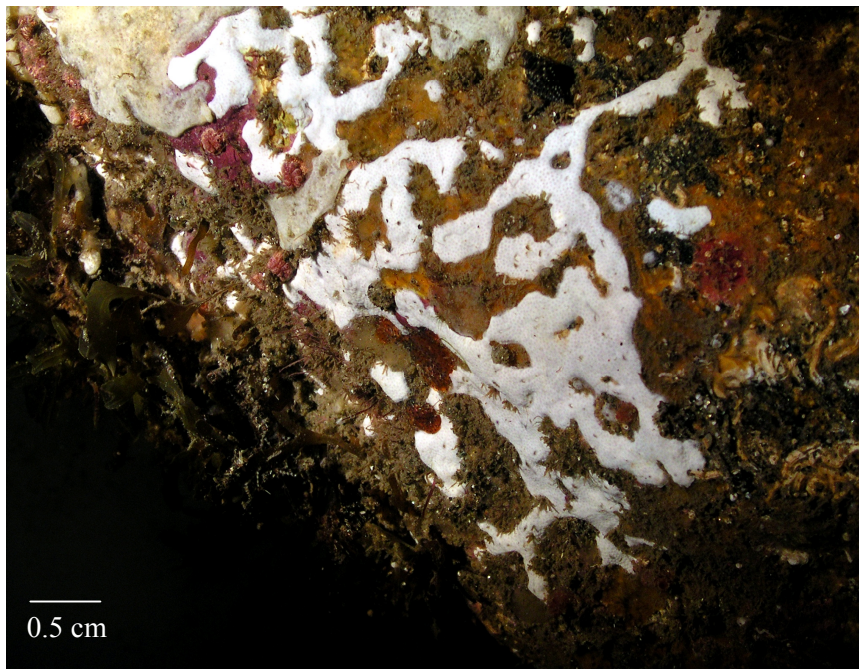
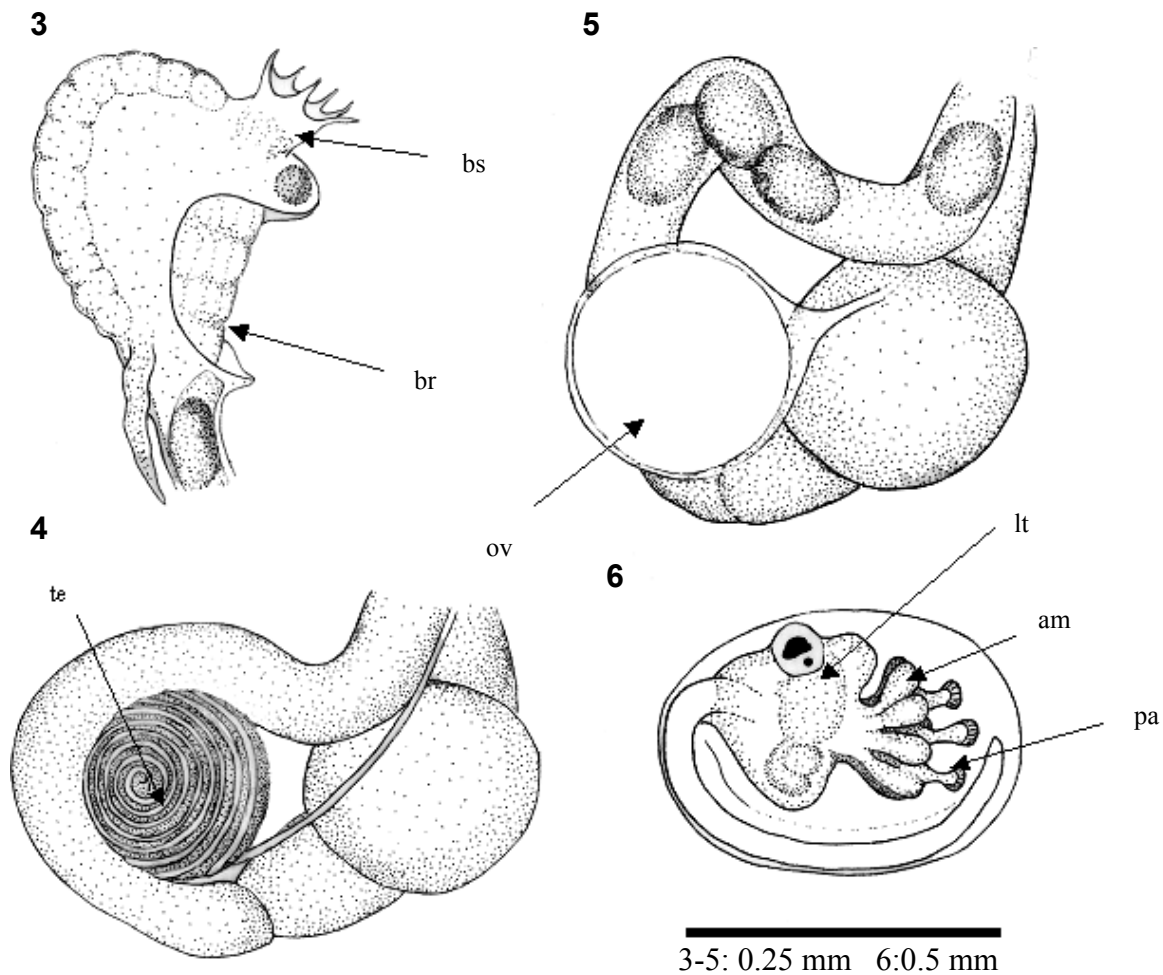


Figure 2. External appearance of *Didemnum* sp n1.

Internal structure – Zooids yellow or orange, less than 1 mm long. Branchial siphon short, with six lobes, atrial aperture a wide cleft exposing much of the branchial sac. Stigmata difficult to see clearly. Muscular process stout, about half as long as the thorax (Fig. 3). Esophageal neck always surrounded by spicules. Testis undivided, with 7 to 9 counterclockwise spiral turns (Fig. 4). Ovary with a single large egg (Fig. 5), never observed occurring simultaneously with testis. Larval trunk about 0.5 mm long, three papillae (pa), four pairs of ampullae and the tail wound halfway around the trunk (Fig. 6).



Figs. 3. Thorax. 4. Male abdomen. 5. Female abdomen. 6. Larvae of *Didemnum* sp. n1, where: bs=branchial siphon, br=branchial sac, te=testis, ov=ovary, lt=larval trunk and am=ampullae.



#### 2.2.4. DISCUSSION

*Didemnum* sp. n1 is distinct from all others of the genus because of the unique structure of its spicules, which form a tetrahedron. Kott (2001), in her extensive monograph of Australian didemnids, remarked that “generally they are spherical (stellate, mulberry-like, globular or burr-like) composed of tightly packed radial rods of calcium carbonate.

Van Name (1945) illustrated groups of spicules that varied in form from stellate to spherical, belonging to different colonies of whitish tropical Atlantic didemnids assembled by him under the name *Didemnum candidum* Savigny, 1816. Although tetrahedral spicules occur in *Echinoclinum verrili* Van Name, 1902, they differ from the ones of the new species by having the four apices extended into elongated points (Van Name, 1945).

In the general appearance of its colonies and the structure of its zooids and larvae, the new species is similar to certain other Tropical Atlantic species such as *D. ahu* Monniot & Monniot, 1987 and *D. speciosum* (Herdman, 1886), which also occur at São Sebastião (Rodrigues & Rocha, 1993; Rocha & Monniot, 1995).

#### 2.2.5. ACKNOWLEDGMENTS

We thank the following institutions for providing logistical support: Centro de Biologia Marinha of the Universidade de São Paulo and the Departamento de Microscopia Eletrônica and Departamento de Zoologia, both of the Instituto de Biologia at UNICAMP. One of us (GMD) also thanks his advisor Dr. Luiz Francisco Lembo Duarte for helpful suggestions and Leonardo Querobim Yokoyama for help with electron microscopy. GMD

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### **2.3. *DIDEMNUM* sp. n2**

RUNNING HEAD: A new didemnid species from Brazil

TITLE: *Didemnum* sp. n2 sp. nov., a new *Didemnum* (Tunicata: Ascidiacea) species from southeastern Brazil.

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### 2.3.1. ABSTRACT

A new species of *Didemnum* was discovered during a recent biodiversity survey of Praia Grande, São Sebastião. This species forms small, thin colonies in the intertidal zone and grows mainly on the undersurfaces of rocks. The species has a peculiar external aspect in which the arrangement of the spicules on the superficial layer of the tunic gives the colonies a punctuated appearance.

### 2.3.2. INTRODUCTION

Species of the genus *Didemnum* Savigny, 1816 are often whitish with small zooids and few morphological characters that allow their distinction.

This paper describes a new species of *Didemnum* found at Praia Grande (23°49'23" S; 45°25'01" W) and Praia Preta (23°49'29" S; 45°24'83" W), São Sebastião, in the State of São Paulo, Brazil, during a study of the composition, richness and diversity of the intertidal compound ascidian fauna (Dias & Duarte, in prep.). The specimens were collected from under boulders exposed at low tide. Compound ascidians from this region have been described by Rocha (1991), Rocha & Monniot (1993) and Rodrigues & Rocha (1993).

Vouchers specimens of the examined material will be deposited in the Museu de Zoologia, Universidade de São Paulo, São Paulo, Brazil (MZUSP).

### 2.3.3. DESCRIPTION

*Didemnum* sp. n2 new species

(Figures 1-6)

Locality – Brazil, São Paulo Estate, Praia Grande, collected by Gustavo Muniz Dias.

Holotype – Two colonies in the MZUSP collected on 27/01/2003.

Paratypes – Two colonies in the USNM collected on 27/01/2003.

Diagnosis – Colonies thin, encrusting, with a moderate concentration of spicules on the superficial layer of the tunic, except over the orange thorax, where they are restricted to a small area over the branchial siphon. This arrangement gives the colonies a spotted aspect. The zooids have a brachial siphon with six very short lobes and a testis divided into two distinct glands.

Etymology – The specific name refers to the external appearance of the colonies (Fig. 1).

External appearance - Colonies very thin, less than 1 mm thick, sheet-like, never observed with more than 7 cm across. Color whitish, sometimes slightly yellow, always with a moderate concentration of spicules on the upper layer of the tunic, except over the orange thorax where they are restricted to a small area over the branchial siphon, thus giving the colonies a punctuated aspect (Fig. 1). Spicules are either spherical of moderate size, around

30  $\mu\text{m}$  across, with cylindrical rays (Fig. 2) or small and globular, around 10  $\mu\text{m}$  across, with irregular rays (Fig. 3). The latter spicule shape is about 1/3 as abundant as the former.



Figure 1. External appearance of *Didemnum* sp. n2.

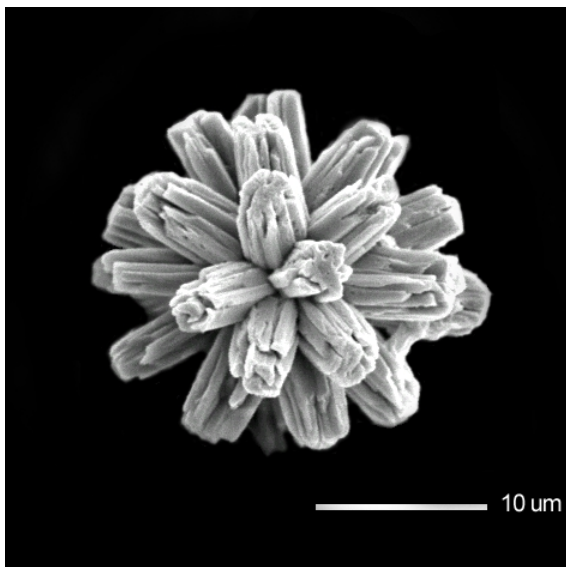


Figure 2. Electron microscopy of *Didemnum* sp. n2 spicule.

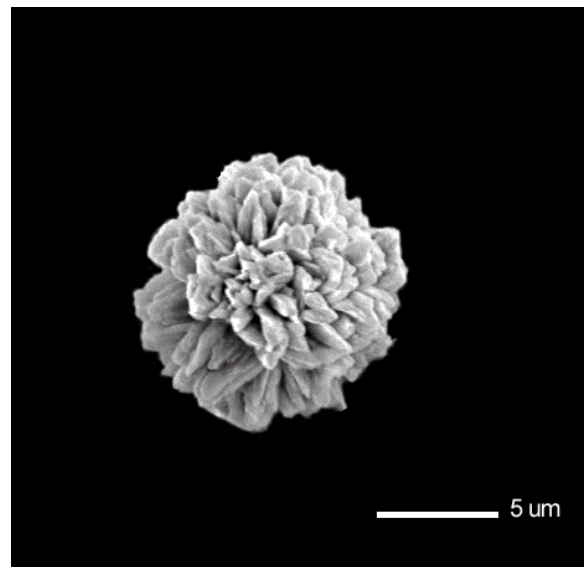
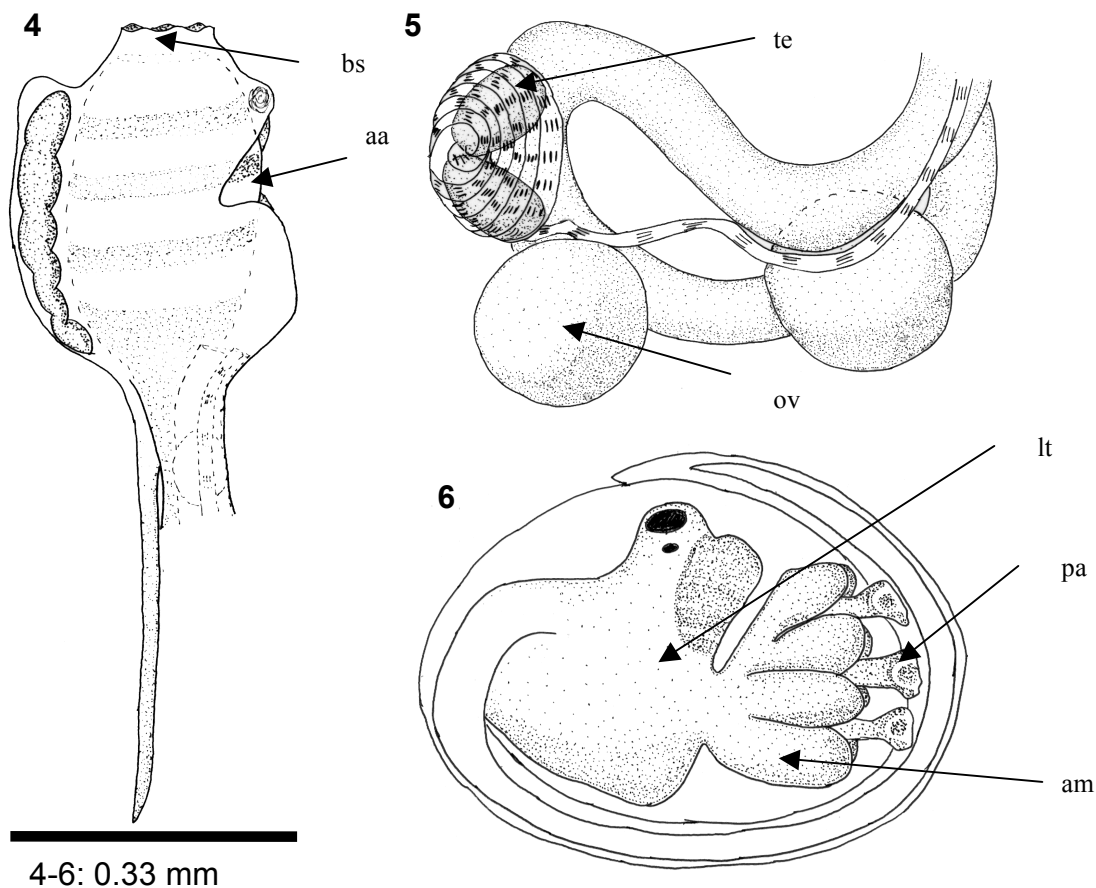


Figure 3. Electron microscopy of *Didemnum* sp. n2 spicule.

Internal structure – Zooids up to 1.3 mm long with an orange thorax and yellow or whitish abdomen. Branchial siphon short, with six small lobes, atrial aperture a cleft of variable size with no languet, often exposing the second and third rows of stigmata. Stigmata difficult to see clearly. Muscular process, about one and a half times as long as the thorax (Fig. 4). Testis on the external face of the digestive tract, divided into two distinct glands with 4-7 anticlockwise spiral turns. Ovary yellowish, with a single large egg, occurring simultaneously with the testis on its inferior portion (Fig. 5). Larval trunk a little less than 0.8 mm long, with three papillae, four pairs of ampullae and the tail winds three quarters of the way around the trunk (Fig. 6).



Figs. 4. Thorax. 5. Abdomen. 6. Larvae of *Didemnum* sp. n2, where bs=branchial siphon, aa=atrial aperture, ov=ovary, te=testis, lt-larval trunk, pa=papillae, am=ampullae.

#### 2.3.4. DISCUSSION

*Didemnum* sp. n2 is distinct from all others of the genus because of the unique distribution of the spicules, which are concentrated on its superficial layer and absent over the orange thorax, except for a small amount over the branchial siphon. *Didemnum* sp. n2 has a testis divided into two glands, as is common in a few species of the genus. The only species with the testis divided into two lobes recorded from the Brazilian coast is that early described as *Didemnum lutarium*, which has milky white external appearance and no spicules on the superficial layer of the tunic (Rocha & Monniot, 1995), thus distinguishing it from the new species described here. *Didemnum* sp. n2 has larger muscular process, zooids and larvae than those of *Didemnum lutarium*, and the orange thorax is responsible for its external appearance. Globular-shaped spicules were not described by Rocha & Monniot (1995) or Van Name (1945) for *Didemnum lutarium*.

Another species with testis divided in two glands described for the Tropical Atlantic Ocean is *Didemnum duplicatum*. It shares innumerable characters with the new species *Didemnum* sp. n2, for example, the pigmentation and size of zooids, the size of spicules and colonies, the small lobes of the branchial siphon and the absence of a languet. Although, *Didemnum* sp. n2 shows a peculiar distribution of spicules and a globular form of spicule not mentioned for *Didemnum duplicatum* in its original description for Guadeloupe (Monniot, 1983). This last one shows a trapezoid stomach and an intestine divided in three regions, while *Didemnum* sp. n2 shows a circular stomach and undivided digestive tract. The number of the vas deferens turns and the size of larvae are larger in *D. duplicatum*, and the ampullae are duplicated, while in *Didemnum* sp. n2 these are simple.



Differences in zooids size and pigmentation, format of the tract digestive and ampullae, larvae size, the spicule format and distribution justify the description of *Didemnum* sp. n2 as a new species.

### 2.3.5. ACKNOWLEDGMENTS

The author thanks the following institutions for providing logistical support: Centro de Biologia Marinha of the Universidade de São Paulo and the Departamento de Microscopia Eletrônica and Departamento de Zoologia, both of the Instituto de Biologia at UNICAMP. The author also thanks his advisor Dr. Luiz Francisco Lembo Duarte and Sérgio de Almeida Rodrigues (*in memoriam*) for their helpful suggestions, Leonardo Querobim Yokoyama for help with electron microscopy, and CAPES for providing a graduate (master degree) studentship.

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## Capítulo 3

**Estrutura genética de *Symplegma rubra* Monniot, C. 1972.**



RUNNING HEAD: Genetic variability in *Symplegma rubra*

TITLE: Genetic structure of *Symplegma rubra* Monniot, C. 1972.

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### 3.1. ABSTRACT

Pelagic larvae are the most important mechanism for maintaining the gene flow among populations of sessile marine invertebrates. Colonial ascidians consist, exclusively, of brooding species, with lecithotrophic larvae that have a limited dispersal. As a result, there is a marked differentiation among populations. In this work we used allozyme electrophoresis to assess the genetic variation in four populations of *Symplegma rubra*, a colonial ascidian frequently found in the intertidal zone of southeastern Brazil. High variability was found at three of the four sites sampled, the exception being Praia Grande in the State of Rio de Janeiro. At this site, there was a great preponderance of clones, which possibly reflected the enclosed nature of the location and its low water circulation that could reduce the dispersal capabilities of these animals. *Symplegma rubra* did not conform to expectations for random mating (Hardy-Weinberg equilibrium). As a result, there was a deficit of heterozygotes. This deficiency was more related to the small population size than to inbreeding processes, since that  $F_{is}$  analysis per locus, revealed a deficiency of heterozygotes at only one locus. The greatest variation in allele frequency was found for *Pgi*. Analyses of genetic variability revealed moderate differentiation among the populations ( $F_{ST}= 0.051$ ), which was unexpected for a species with a low dispersal capability. Rafting, a frequently underestimated means of dispersal, may be the main mode of maintain the gene flow in this species over large areas, since colonies of *Symplegma rubra* are frequently seen growing on drift material and there is no evidence that the larva survive for long in the plankton.

### 3.2. INTRODUCTION

The movement of individuals from one location to another affects many ecological and genetic properties of populations (Burton, 1983; Palumbi, 1994; Hanski & Gilpin, 1997; Bohonak, 1999; Ayre & Hughes, 2000). The rate of gene movement from one population to another contributes to determine the possibility of local adaptation and to adaptative evolution in complex landscapes (Whitlock & McCauley, 1999). Gene flow can result from the movement of gametes (Grosberg, 1991; Yund, 1995), the extinction and recolonization of entire populations, or the movement of extranuclear segments of DNA (Slatkin, 1985).

On a local scale, marine organisms are typically restricted to patches of favorable habitats. Depending on the distances among the patches, populations of sedentary invertebrates may be geographically isolated from conspecific populations and may show genetic differentiation in response to local selective pressures and random drift. (Burton, 1983). For these animals, pelagic larvae provide a potent means of dispersal among conspecific populations, and can determine the species abundance and life histories (Strathmann, 1974; Palmer & Strathmann, 1981; Burton, 1983; Hedgecock, 1986; Hines, 1986; Yamada, 1989).

Population differentiation in marine invertebrates with pelagic larvae can arise from either physical or biological barriers to larval dispersal or from the differential survival or fecundity of immigrants (Hedgecock, 1986; Schilthizen & Lombaerts, 1994).

A growing body of evidence suggests that clonal organisms (ascidians, bryozoans, sponges and hydroids) typically exhibit patterns of dispersal, population structures, and genotype-environment interactions that may differ from those of many of the better-studied “sexual” species (Ayre *et al.*, 1991). These animals disperse by asexual and sexual means, and may often disperse farther by the former, although their larvae normally show a restricted dispersal capacity when compared to aclonal species (Jackson, 1986; Worcester, 1994).

Because of the preponderance of asexual reproduction and short-distance larval dispersal among clonal animals, local populations of clonal species may be dominated by large numbers of closely related individuals (Jackson, 1986). The recruitment of offspring in parental habitats may increase inbreeding, that will lead to the loss of heterozygosity and a decrease in the overall population fitness (Grosberg, 1987; Miller, 1998).

Ascidians consist of colonial and solitary species, with both forms having non-feeding larvae. Aclonal species are generally oviparous, with a pelagic development lasting up to 6 days (average of 1-2 days) whereas most clonal ascidians are viviparous, with a free larval existence of less than 3 h (Millar, 1971; Ayre *et al.*, 1997).

Electrophoretic allozyme patterns have been used to assess the genetic structure of ascidians. Aron & Solé-Cava (1991) observed a high level of gene variation between sympatric populations of two different morphotypes of *Botrylloides nigrum* and suggested that they were distinct species. Using the same method, Dalby (1997) detected dimorphism in the ascidian *Pyura stolonifera*. Allozyme electrophoresis has also been used to elucidate population structures and systematic problems in other groups of animals and plants.

The coloniality has evolved more than once in the Class Ascidiacea, with representatives in the orders Enterogona and Pleurogona. In the latter order, only the family

Styelidae has colonial species (Wada *et al.*, 1992; Stach & Turbeville, 2002). This family is represented by 16 species on the coast of the State of São Paulo in southeastern Brazil. Of these species, those of the genus *Symplegma* have had their morphological taxonomy studied by Couto (2003).

*Symplegma rubra* is easily recognized on rocks by its homogeneous red, pink or yellow coloration and a red ring that unites both siphons (inconspicuous in some red colonies). According to Couto (2003), there is no evidence of morphological differentiation among the morphs, with all colonies having a continuous peripharyngeal band, a double V-shaped dorsal lamina, zooids 2.5-4mm long, 12-13 rows of stigmata, frequently without simultaneous male and female gonads and larvae 1.5mm long.

In this work we examined the variability in allozyme and the genetic structure of four populations of *Symplegma rubra* in southeastern Brazil.

### 3.3. MATERIAL AND METHODS

#### 3.3.1. Sampling sites and treatment

*Symplegma rubra* C. Monniot, 1972 is a locally abundant species with a range that extends along the coast of Tropical America. Specimens were collected from pier pilings at Praia Deserta (PD), São Sebastião-SP (23°46'89" S; 45°23'65" W), on a rocky shore at Praia Grande (PG<sub>SP</sub>), São Sebastião-SP (23°49'23" S; 45°25'01" W), from a pier pilings at Praia Grande (PG<sub>RJ</sub>), Angra dos Reis-RJ (23°01'33" S, 44°20'36" W) and on a rocky shore at Praia do Forte (PF), Cabo Frio-RJ (22°53'10" S, 42°00'50" W) (Figure 1).



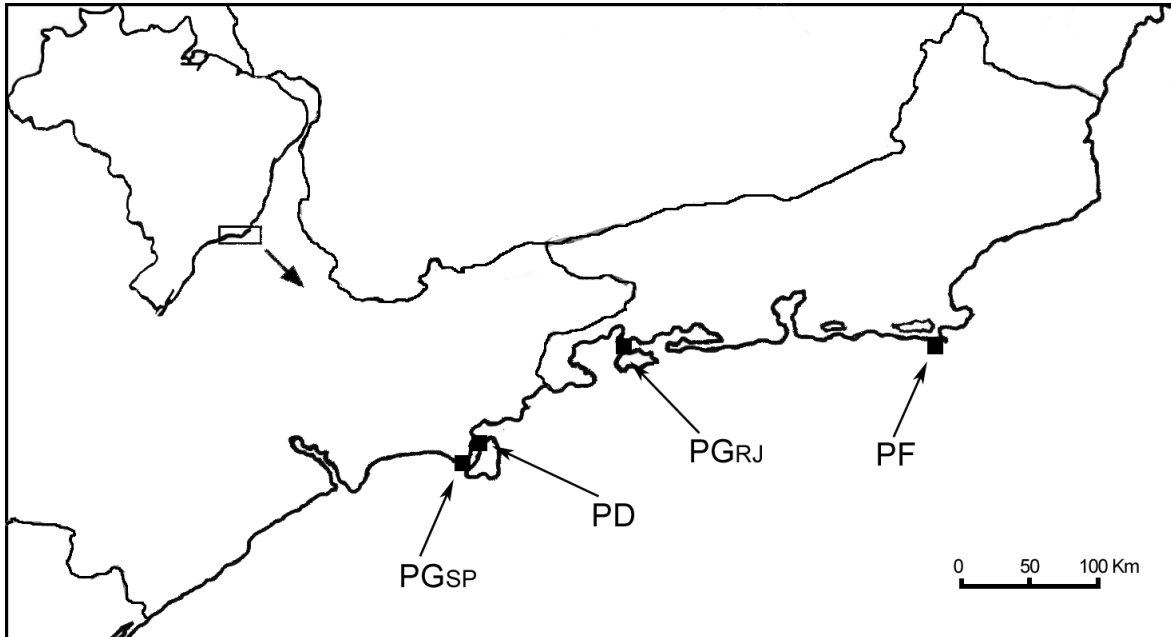


Figure 1. Sampling locations in southeastern Brazil, where PG<sub>SP</sub>=Praia Grande, São Sebastião-SP, PD=Praia Deserta, São Sebastião-SP, PG<sub>RJ</sub>=Praia Grande, Angra dos Reis-RJ, PF=Praia do Forte, Cabo Frio-RJ.

At each collection 30 small fragments (1-2 cm<sup>2</sup>) were removed from different colonies in the intertidal and subtidal zones at depths of 0 to 3 m. Closed colonies were not collected in order to reduce the possibility of gathering clones. The color of the specimens was recorded, although the small number of orange, yellow and pink specimens made it impossible to analyze the genetic basis of the coloration. The collected material was immediately frozen and stored in liquid nitrogen until tissue extraction in the laboratory.

### 3.3.2. Allozyme electrophoresis

Tissue extracts were prepared by homogenizing small pieces of frozen tissue in 33 µL of 1.22 M Tris-citrate buffer, pH 8.0, containing 0.05% (v/v) β-mercaptoethanol. The

extracts were then applied to Whatman #3 paper wicks (1.0x0.3 cm), which were loaded onto horizontal 8.5% starch gels. All colonies within each of four populations were assayed for 12 enzyme systems resulting in 15 polymorphic and 3 monomorphic enzyme-encoding loci in four buffer systems (Table 1).

Table 1. Encoding enzymes, loci and systems studied in *Symplegma rubra*.

Enzimas	ECN	Loci	System
Pyrophosphatase	3.6.1.1	<i>Pp</i>	(b)
Alcohol dehydrogenase	1.1.1.1	<i>Adh</i>	(a)
Hexokinase	2.7.1.1	<i>Hk</i>	(a)
Leucine aminopeptidase	3.4.11.1	<i>Lap</i> <sup>-1</sup>	(c)
		<i>Lap</i> <sup>-2</sup>	(c)
Esterase	3.1.1.1	<i>Est</i> <sup>-1</sup>	(c)
		<i>Est</i> <sup>-2</sup>	(c)
Isocitrate dehydrogenase	1.1.1.42	<i>Idh</i>	(b)
Malate dehydrogenase	1.1.1.37	<i>Mdh</i>	(a)
Adenylate kinase	2.7.4.3	<i>Ak</i>	(b)
Acid phosphatase	3.1.3.2	<i>Acph</i>	(a)
Phosphoglucomutase	2.7.5.1	<i>Pgm</i> <sup>-1</sup>	(b)
		<i>Pgm</i> <sup>-2</sup>	(b)
Peptidase-leucyl-alanine	3.4.13.9	<i>Pep</i>	(b)
Glucose phosphate isomerase	5.3.1.9	<i>Pgi</i> <sup>-1</sup>	(d)
		<i>Pgi</i> <sup>-2</sup>	(d)
		<i>Pgi</i> <sup>-3</sup>	(d)
		<i>Pgi</i> <sup>-4</sup>	(d)

(a) Electrode: 0.3 M boric acid, 60 mM NaOH, pH 7.0. Gel: 10 mM Tris, pH 8.5 (Shaw & Prasad, 1970)

(b) Electrode: 0.04 M citric acid and N (3 aminopropyl) morpholine adjusted to pH 6.1. Gel: electrode solution diluted 1:20 (Clayton & Tetriak, 1972)

(c) Electrode: 10 mM lithium hydroxide, 90 mM boric acid, 3 mM EDTA, pH 8.0. Gel: electrode solution diluted 1:10.

(d) Electrode: 0.60 M boric acid, adjusted to pH 8.0. Gel: 0.15 M Tris, pH 8.5, 0.01 M citric acid.

### 3.3.3. Data analysis

#### 3.3.3.1. *Variability within populations*

The genetic variability in each sample was inferred through: (a) the percentage of polymorphic loci (99% criteria), (b) the mean number of alleles per locus, and (c) the mean heterozygosity per locus, observed and expected (gene diversity). Deviations from the expected frequencies for Hardy-Weinberg equilibrium were expressed as Wright's (1978) fixation index ( $F_{IS}$ ) and tested by a randomization procedure using the software Fstat 1.2 (Goudet, 1995).

The observed ( $G_o$ ) and expected ( $G_e$ ) multilocus genotypic diversities were calculated for each population according to Stoddart & Taylor (1988). Since no genotypes were predicted to occur more than once in any population,  $G_e$  was equal to  $N$  in all populations (Burnett *et al.*, 1995). The departure of  $G_o:G_e$  from unity provided an index of the combined effects of departures from single-locus Hardy-Weinberg equilibrium and multi-locus linkage equilibrium. A population with high asexual recruitment should have a low  $G_o:G_e$  ratio (Ayre *et al.*, 1997).

The number of unique multilocus genotypes ( $N_g$ ) was counted. The  $N_g:N$  ratio provided a maximum estimate of the contribution of asexual reproduction to localized recruitment. Since only a small portion of the genome can be sampled electrophoretically, colonies that are genotypically identical may still be nonclonemates (Ayre & Hughes, 2000).

### 3.3.3.2. Genetic differentiation among populations

The relationships among samples were evaluated using Wright's hierarchical F-statistics (Wright 1978) estimated according to theta ( $\theta$ ) of Weir and Cokerham (1984), the gene flow estimative ( $N_e m$ ), and unbiased genetic distance matrices ( $D$ ) (Nei, 1978). The average  $F_{ST}$  was estimated with Jackknife permutations of the loci and the confidence interval (95%) was determined by 5000 bootstrap resamplings using the software Fstat 1.2 (Goudet, 1995). This standardized genetic variance ( $F_{ST}$ ) was used to infer the extent of population subdivision by quantifying the level of allelic variation among local populations (Ayre *et al.*, 1997), and is the most adequate estimate for the "random populations" model (Black and Krafur 1985). A matrix of genetic distances was used for cluster analysis by the method UPGMA with the software TFPGA (Miller, 1997).

To assess the importance of isolation on genetic differentiation, the correlation between genetic structure (by pairwise  $F_{ST}$ ) and geographic distance matrices was investigated using a Mantel test (Hutchinson and Templeton 1999) done with the software Genetix v. 4.02 (Belkhir *et al.*, 2001).

### 3.3.3.3. Linkage-disequilibria

For all analyses, we assumed that each locus provided independent information about the genetic structure of the *Symplegma rubra* populations. The diallelic pairwise measure of linkage-disequilibria was thus calculated by the software Genetix v. 4.02

(Belkhir *et al.*, 2001). Since no significant linkage-disequilibria were observed, all loci were treated as unlinked.

### 3.4. RESULTS

#### 3.4.1. Variability within populations

The allele frequencies and sample sizes for all of the polymorphic loci at each collection site are shown in Table 2. The loci  $Pgm^{-2}$ ,  $Pep$ ,  $Pgi^{-1}$  were monomorphic. At the population level, the mean percentage of polymorphic loci (P) was 65.3%, the number of alleles per polymorphic locus ( $A_p$ ) was 2.57, and the expected heterozygosity ( $H_e$ ) was 0.127. The number of alleles at the species level was higher than that found in each sampling area, indicating the presence of unique alleles per population. The percentage of polymorphic loci per area was 72.3-83.3%, except for Praia Grande-RJ (PG<sub>RJ</sub>), where it was <30%. The  $Ge:Go$  ratios indicated that asexual reproduction resulted in the multiple occurrence of genotypes in the populations, with the lowest value at Praia Grande-RJ (PG<sub>RJ</sub>) (Table 3). Praia Deserta (PD) had the highest number of alleles, percentage of polymorphic loci and observed and expected heterozygosity.

Significant departures from Hardy-Weinberg expectations were observed at all sites, except Praia Grande-RJ (PG<sub>RJ</sub>). In all cases, this departure resulted from a deficiency of heterozygotes ( $p < 0.05$ ) (Table 3).

Table 2. Allele frequency for the 15 polymorphic enzyme loci

	<i>Pp</i>				<i>Lap</i> <sup>-1</sup>				<i>Lap</i> <sup>-2</sup>					
	n	0.78	1.00	1.29	n	0.63	0.72	1.00	1.11	n	0.91	0.96	1.00	1.03
<b>PD</b>	27	-	0.91	0.09	12	0.04	0.08	0.75	0.13	19	0.03	0.10	0.87	-
<b>PG<sub>SP</sub></b>	30	-	0.97	0.03	18	-	-	1.00	-	8	0.13	0.06	0.81	-
<b>PG<sub>RJ</sub></b>	17	-	1.00	-	24	-	-	0.98	0.02	24	-	0.04	0.96	-
<b>PF</b>	23	0.04	0.96	-	24	-	-	0.98	0.02	16	0.03	0.03	0.91	0.03

	<i>Adh</i>				<i>Hk</i>			<i>Est</i> <sup>-1</sup>						
	n	0.93	1.00	1.09	1.16	n	0.97	1.00	1.07	n	0.75	0.82	1.00	1.13
<b>PD</b>	29	0.03	0.97	-	-	30	0.07	0.93	-	30	0.02	0.03	0.95	-
<b>PG<sub>SP</sub></b>	28	0.03	0.95	-	0.02	30	-	0.98	0.02	30	-	-	0.97	0.03
<b>PG<sub>RJ</sub></b>	25	-	0.96	0.04	-	30	-	0.98	0.02	28	-	0.02	0.98	-
<b>PF</b>	30	0.03	0.93	0.02	0.02	29	0.03	0.97	-	30	-	0.13	0.75	0.12

	<i>Est</i> <sup>-2</sup>			<i>ldh</i>				<i>Acph</i>					
	n	0.85	1.00	1.10	n	0.77	1.00	1.18	1.59	n	0.77	1.00	1.07
<b>PD</b>	30	0.03	0.97	-	28	0.04	0.96	-	-	27	0.05	0.91	0.04
<b>PG<sub>SP</sub></b>	30	-	0.90	0.10	30	-	0.95	0.02	0.03	18	0.06	0.88	0.06
<b>PG<sub>RJ</sub></b>	30	0.02	0.98	-	25	0.02	0.96	0.02	-	18	-	1.00	-
<b>PF</b>	30	-	0.88	0.12	28	0.02	0.98	-	-	18	0.03	0.97	-

	<i>Mdh</i>					<i>Pgm</i> <sup>-1</sup>			<i>Ak</i>						
	n	0.95	1.00	1.07	1.13	n	0.78	1.00	1.20	n	0.69	0.81	1.00	1.30	1.66
<b>PD</b>	30	0.02	0.93	0.03	0.02	25	0.12	0.86	0.02	29	-	0.04	0.81	0.12	0.03
<b>PG<sub>SP</sub></b>	29	0.09	0.88	0.03	-	25	0.08	0.84	0.08	27	-	0.07	0.93	-	-
<b>PG<sub>RJ</sub></b>	26	0.04	0.85	0.11	-	23	0.02	0.98	-	26	0.02	0.04	0.92	0.02	-
<b>PF</b>	28	-	0.93	0.07	-	26	0.02	0.98	-	29	-	0.02	0.95	-	0.03

	<i>Pgi</i> <sup>-2</sup>				<i>Pgi</i> <sup>-3</sup>				<i>Pgi</i> <sup>-4</sup>					
	n	0.58	1.00	1.52	1.85	n	0.77	0.85	1.00	1.18	n	0.92	1.00	1.05
<b>PD</b>	17	0.12	0.70	0.18	-	30	-	-	0.98	0.02	27	0.06	0.81	0.13
<b>PG<sub>SP</sub></b>	18	-	0.86	0.08	0.06	29	-	-	0.97	0.03	17	0.09	0.91	-
<b>PG<sub>RJ</sub></b>	20	0.03	0.77	0.17	0.03	29	-	0.02	0.95	0.03	22	0.18	0.77	0.05
<b>PF</b>	18	-	0.36	0.58	0.06	30	0.03	0.02	0.92	0.03	15	0.07	0.86	0.07

Table 3. Intrapopulational genetic attributes of *Symplegma rubra* at the four sampling sites. \* = significant ( $p < 0.05$ ) departure from Hardy-Weinberg expectations.  $F_{IS}$  = Wright's (1978) fixation index.  $Ge$  = expected multilocus genotypic diversities.  $Go$  = observed multilocus genotypic diversities.  $N_g$  = number of unique multilocus genotypes.

Sample	N	Mean number of alleles per locus (Ap)	Polymorphic loci (%)	Mean heterozygosity		$F_{IS}$	$N_g:N$	$Ge:Go$
				Observed (Ho)	Expected HdyWbg (He)			
PD	30	2.8	83.3	0.109	0.162	0.347*	0.90	0.789
PG <sub>SP</sub>	30	2.4	77.8	0.079	0.121	0.366*	0.77	0.469
PG <sub>RJ</sub>	30	2.4	27.8	0.079	0.093	0.166	0.53	0.300
PF	30	2.67	72.3	0.072	0.133	0.475*	0.80	0.682
Mean	30	2.57	65.3	0.085	0.127	-	-	-
Species level	120	3.67	83.3	0.083	0.133	-	-	-

Except for the *Mdh* locus that showed a heterozygote deficiency at Praia Grande-RJ (PG<sub>RJ</sub>) and Praia Grande-SP (PG<sub>SP</sub>) ( $p < 0.05$ ), the observed genotype frequencies for the other polymorphic loci conformed to Hardy-Weinberg predictions (Table 4).

Table 4. Departures from Hardy-Weinberg equilibria expressed as a fixation index ( $F_{IS}$ ) for each of the 15 enzyme-encoding loci analyzed at four sites in southeastern Brazil. \*  $p < 0.05$ . n.d.- test not done because the degree of freedom was zero.

	PD	PG <sub>SP</sub>	PG <sub>RJ</sub>	PF
<b><i>Pp</i></b>	0.36	-0.02	n.d.	1.00
<b><i>Adh</i></b>	1.00	0.66	-0.02	0.49
<b><i>Hk</i></b>	1.00	0.00	0.00	1.00
<b><i>Lap</i><sup>-1</sup></b>	0.04	n.d.	0.00	0.00
<b><i>Lap</i><sup>-2</sup></b>	0.35	-0.11	-0.02	-0.03
<b><i>Est</i><sup>1</sup></b>	0.66	-0.02	0.00	0.36
<b><i>Est</i><sup>2</sup></b>	-0.02	0.64	0.00	0.84
<b><i>Idh</i></b>	-0.02	-0.02	-0.01	0.00
<b><i>Mdh</i></b>	-0.03	0.85*	1.00*	1.00
<b><i>Ak</i></b>	0.49	-0.06	0.48	-0.02
<b><i>Acph</i></b>	-0.06	0.48	n.d.	0.00
<b><i>Pgm</i><sup>-1</sup></b>	0.21	0.45	0.00	0.00
<b><i>Pgi</i><sup>-2</sup></b>	0.51	0.79	-0.20	0.70
<b><i>Pgi</i><sup>-3</sup></b>	0.00	1.00	-0.02	-0.04
<b><i>Pgi</i><sup>-4</sup></b>	0.55	-0.07	0.03	0.47

### 3.4.2. Genetic differentiation among populations

A high degree of divergence in the allelic frequency was observed among the four populations ( $p < 0.0001$ ). The loci *Est*<sup>-1</sup>, *Lap*<sup>-1</sup> and *Pgi*<sup>-2</sup> showed the highest differentiation among the sampling areas, with the last of these loci showing inverted frequency at Praia do Forte (PF) (Figure 2). The mean  $F_{ST}$  value for *Symplegma rubra* was 0.051 (range: 0.003-0.169) and  $N_e m = 4.7$ , indicating moderately structured populations.

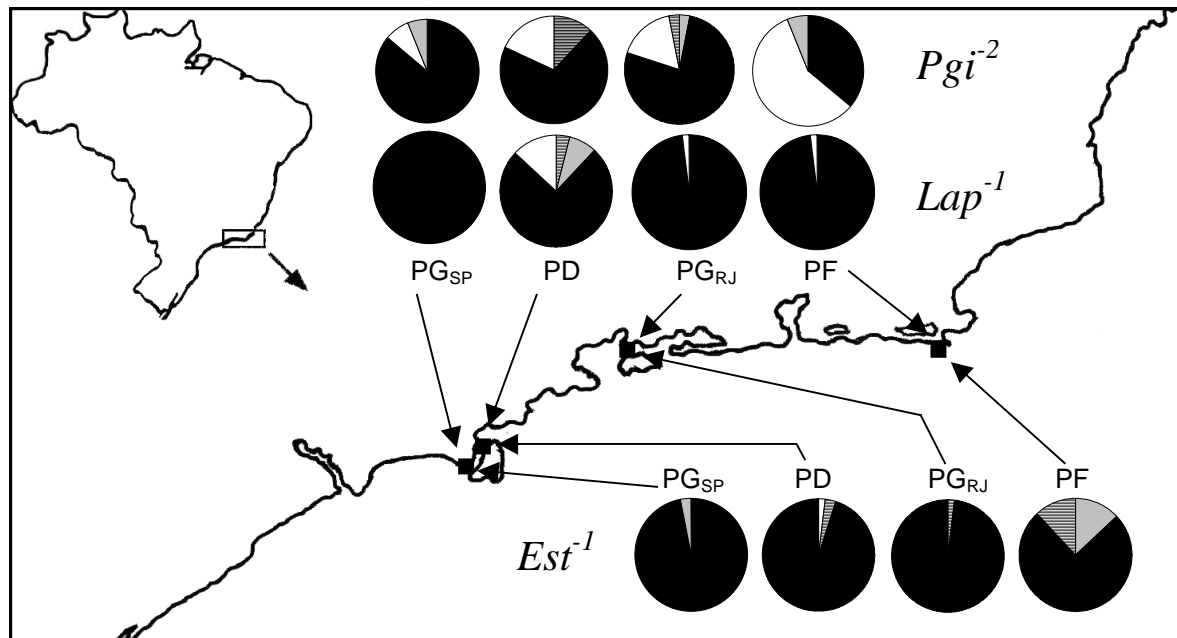


Figure 2. Pie diagrams illustrating the variation in allele frequencies among the sampling areas for the three *Symplegma rubra* loci with the highest  $F_{ST}$  values, where PG<sub>SP</sub>=Praia Grande, São Sebastião-SP, PD=Praia Deserta, São Sebastião-SP, PG<sub>RJ</sub>=Praia Grande, Angra dos Reis-RJ, PF=Praia do Forte, Cabo Frio-RJ.

The paired  $F_{ST}$  of the samples showed that Praia Grande-SP (PG<sub>SP</sub>) did not differ significantly from Praia Grande-RJ (PG<sub>RJ</sub>) and Praia Deserta (PD). Curiously, Praia Deserta (PD), located between PG<sub>SP</sub> and PG<sub>RJ</sub> was genetically different from the latter. Praia do Forte (PF), the most distant site, was the only one that differed from all of the other three beaches (Table 5).



Table 5.  $F_{ST}$  per pair of samples. \*  $p < 0.05$ .

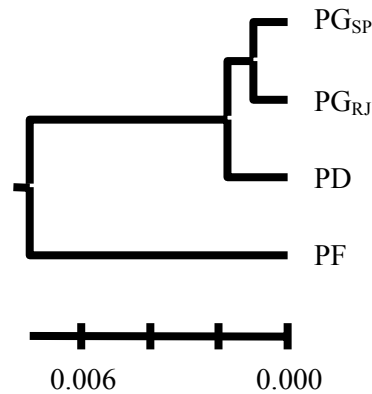
	<b>PG<sub>SP</sub></b>	<b>PG<sub>RJ</sub></b>	<b>PF</b>
<b>PD</b>	0.01781	0.02225*	0.06775*
<b>PG<sub>SP</sub></b>	-	0.01671	0.09958*
<b>PG<sub>RJ</sub></b>	-	-	0.08229*

Nei's genetic distance ( $D$ ) between the four sites ranged from 0.002 between Praia Grande-RJ and Praia Grande-SP to 0.018 between the latter and Praia do Forte (Table 6). The UPGMA dendrogram is shown in Figure 3.

The Mantel test revealed that neither genetic structure ( $F_{ST}$ ) nor genetic distance ( $D$ ) was correlated with geographic distance ( $p > 0.05$ ), probably because of the high similarity between Praia Grande-RJ and Praia Grande-SP.

Table 6. Nei's (1978) unbiased genetic distances (above diagonal) and the geographic distances (km) (below diagonal) between populations of *Symplegma rubra*.

	<b>PD</b>	<b>PG<sub>SP</sub></b>	<b>PG<sub>RJ</sub></b>	<b>PF</b>
<b>PD</b>	-	0.004	0.003	0.014
<b>PG<sub>SP</sub></b>	3.86	-	0.002	0.018
<b>PG<sub>RJ</sub></b>	137.63	141.07	-	0.013
<b>PF</b>	360.59	363.07	239.27	-

Figure 3. UPGMA (Sneath & Sokal, 1973) dendrogram of the genetic distances (Nei, 1978) among the four populations of *Symplegma rubra*.

### 3.5. DISCUSSION

#### 3.5.1. Variability within populations and modes of reproduction

The mean number of alleles per locus and the percentage of polymorphic loci were as high or higher than described for other sessile marine invertebrates, including colonial ascidians (Aron & Solé-Cava, 1991; Ayre *et al.*, 1997), despite the small sample size. Moderate expected heterozygosity was still seen. High polymorphism is frequently found among marine invertebrate populations (Solé-Cava & Thorpe, 1991) and may result in considerable malleability of the species under highly variable environmental conditions, especially in the intertidal zone (Powell, 1971 and Levinton, 1973).

The small percentage of polymorphic loci seen at Praia Grande–RJ, probably reflected the lack of Hardy-Weinberg departures at this site, departures that were observed on the other three areas and are common among marine animal populations (Burton, 1983; Johnson & Black 1984; Solé-Cava & Thorpe, 1991; Ayre *et al.*, 1997), especially in colonial species. Grosberg (1991) reported a lack of heterozygotes in a population of *Botryllus schlosseri* in a sheltered marina, and attributed this deficiency to the physical conditions of the site. Curiously, Praia Grande–RJ, the only sampled area with similar characteristics to those described by Grosberg (1991), was an exception to this deficiency.

The deficit in heterozygotes seen here was not associated with the sampling of separate gene pools, since no linkage-disequilibria were detected. The Wahlund effects was also improbable since the colonial ascidians in these areas recruit mainly during the warm months (Rocha, 1991), and shows marked mortality in the cold months (Dias & Duarte, in

prep.), thus reducing the probability of sampling different cohorts simultaneously. Inbreeding processes are common in colonial ascidians, although the low differentiation seen here among the populations of *Symplegma rubra* makes this unlikely. Small heterozygote deficits were recorded only for the *Mdh* locus and could reflect the presence of null alleles or, more likely be due to the small sample size from each population.

Clonal genotypes are common among colonial species and reinforce the importance of asexual reproduction in the population genetic pool (Shick *et al.*, 1979; Ayre, 1983; Burnett *et al.*, 1995; Ayre *et al.*, 1997). The marked variation in this attribute among populations suggested that environmental conditions influenced the asexual:sexual reproduction ratio (Ayre & Hughes, 2000).

The *Ge:Go* value for Praia Grande-RJ was probably underestimated because of its low genetic variability. However, when the colonies with the most common multilocus genotype were scored as non-clone individuals, this area still had the lowest *Ge:Go* ratio, indicating elevated asexual reproduction in this area (Stodart & Taylor, 1988). This sampling site is located in a sheltered bay with low water circulation, which could reduce the colonization and dispersal of ascidians. This site probably also had the most intense competition for space since it was the only one of the four beaches with no bare rocks. Together, these environmental factors could reduce the genotypes to those, which are most resistant, and could account for the high genetic similarity at this site (Slatkin, 1985; Burton, 1986; Neigel, 1999). Further data are needed to confirm this hypothesis.

### 3.5.2. Genetic differentiation among populations and large-scale dispersal

Our data for *Symplegma rubra* do not support the prediction that colonial ascidians show high genetic differentiation among isolated populations.

The largest differentiation at Praia do Forte was probably a combination of its geographical location, as the most distant study area, and of the Falkland current upwelling, that makes the seawater colder in this area (Martin *et al.*, 1989). Temperature is a major factor controlling the geographical distribution of ascidians (Millar, 1971) and differences in thermal stability have been reported for alleles of the *Pgi* locus in gastropods (Wilkins, 1977) and anemones (Hoffmann, 1981). The higher  $F_{ST}$  value for this locus in *Symplegma rubra* suggested that selective pressures could be involved. *Est<sup>-1</sup>* and *Lap<sup>-1</sup>* also showed some differentiation, but the allele frequency at these two loci was not as variable as in *Pgi<sup>-2</sup>* and could be related to the sample size.

The moderate  $F_{ST}$  value, (four times smaller than in similar studies of colonial ascidians) (Ayre *et al.*, 1997), the small genetic distances (Nei, 1978) here, and the lack of correlation between the geographic and genetic distances indicated a low to moderate differentiation and a greater gene flow ( $N_e m$ ) than reported for other colonial ascidians. These factors were sufficient to maintain a relatively high genetic uniformity among the four populations of *Symplegma rubra* separated by over than 360 km. This genetic homogeneity could be the result of similar selection pressures, although the differences in hydrodynamic and physical characteristics of the beaches make this possibility unlikely.

Similar studies have reported a high level of differentiation among closer populations of other marine clonal animals (Russo *et al.*, 1994; Burnett *et al.*, 1995; Ayre *et*

*al.*, 1997; Yund & O'Neil, 2000). The main argument for the relationship between this life form and genetic structure is related to the short larval period in plankton, which limits the species' dispersal. The larvae of colonial ascidians rarely last for more than 24 h in plankton (Millar, 1971), and this is probably also the case for *Symplegma rubra*. Thus, other modes of dispersal whose importance has been underestimated could explain the genetic pattern of this species much better.

Ayre *et al.* (1997) demonstrated that temperate brooding colonial ascidians had an  $F_{ST}$  value (0.20) and an  $N_e m$  (<1.0) that were two orders of magnitude higher than found among broadcast-spawning solitary ascidians. In a similar study, Russo *et al.* (1994) observed marked genetic distinctions between populations of two anemones with different reproductive strategies and larval durations. *Bunodosoma caissarum*, which reproduces sexually and has long-lived planktotrophic larvae, has less structured populations than *Actinia bermudensis*, which reproduces sexually and asexually and has short-lived larvae.

Recent studies have shown that this association between the genetic patterns and life forms is not true for all organisms and that habitat organization can still affect the genetic structure. Thus, Ayre & Hughes (2000) reported that there was no difference between the dispersal capabilities of brooding and broadcast spawner coral species. The sea anemone *Urticina eques* has a larva with a low dispersal capability, but a high genetic similarity between distant populations, features that have been considered adaptations to a sublittoral habitat (Solé-Cava *et al.*, 1994). In agreement with these findings, *Symplegma rubra*, which has a low dispersal capability, showed low to moderate differentiation among its populations.

Adult *Symplegma rubra* are frequently found growing on drift logs, wood and man-made structures. Those are used as a form of transport known as rafting (Jokiel, 1984,

1989). This phenomenon may be important in species distribution patterns. Over distances of a few meters to a few kilometers, larval dispersal via plankton is the most likely means of dispersal (Jackson, 1986), although Worcester (1994) noted that colonies of the ascidian *Botrylloides* sp. growing on broken blades of algae traveled 200 times farther and had a comparable recruitment success relative to swimming larvae.

In conclusion, the results of this study indicate that *Symplegma rubra* can disperse over long distances, most likely through clonal propagules. This finding reinforces the importance of this mode of reproduction for maintaining the gene flow between distant populations. The findings also support the suggestion by Fadlallah (1982) and Jackson (1986) that the macro-scale dispersal of sessile adults in colonial animals is more important than larval dispersal.

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## CONSIDERAÇÕES FINAIS

Este trabalho demonstra que ascídias coloniais no entremarés do litoral de São Sebastião tendem a ser mais abundantes e diversificadas durante os meses de verão;

Mostra que o mediolitoral superior é a região com menor composição, riqueza e diversidade de espécies;

Estabelece que existem diferenças não tão sutis entre os três estratos do entremarés, quanto à estrutura da comunidade estudada;

Ratifica a grande diversidade de ascídias coloniais do entremarés rochoso do litoral norte paulista;

Caracteriza a dinâmica da comunidade de ascídias, cuja composição muda em pequenos intervalos de tempo, com acréscimo no número de espécies no início da primavera;

Descreve duas novas espécies de ascídias coloniais, *Didemnum* sp. n1 e *Didemnum* sp. n2, pertencentes à família Didemnidae;

Demonstra a alta variabilidade genética da ascídia colonial *Symplegma rubra* para o litoral dos Estados do Rio de Janeiro e São Paulo;

Esclarece que existe uma moderada diferenciação genética entre as populações de *Symplegma rubra* analisadas, sugerindo que modos de dispersão como o “rafting” podem ser de grande importância para a manutenção do fluxo gênico;

Verifica para o loco  $Pgi^{-2}$  em *Symplegma rubra*, variações substanciais da frequência alélica entre as áreas estudadas, o que sugere a ação de pressões seletivas relacionadas à temperatura da água do mar.



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## **APÊNDICE**

Descrição das espécies de ascídias coloniais amostradas durante o estudo



## *Polyclinum constellatum* Savigny, 1816

(Figs. A-B)

**Tamanho:** Colônias alcançando 3cm de altura, raramente com mais de 6cm de diâmetro. Zoóides com 5,0 a 8,0mm de comprimento.

**Identificação:** Colônias achatadas em forma de domo, geralmente incrustada com areia na sua camada mais superficial (Fig. A). Túnica lisa, de coloração verde escura (Fig. B), marrom esverdeada ou quase preta.

Zoóide com sífão branquial com 6 lobos e atrial com uma grande lingüeta na sua porção dorsal. Gônadas localizadas no pós-abdômen saculiforme. Larva com 1,3mm de comprimento, 3 papilas e 4 pares de ampolas.

**Comentários:** Em função da sua espessura é fácil perceber uma colônia de *Polyclinum constellatum*. Na sua descrição original as colônias apresentam os zoóides organizados em torno de uma cloaca comum formando numerosos sistemas. Entretanto, poucos representantes desta espécie no litoral brasileiro apresentam este tipo de organização dos zoóides.

**Habitat:** Região entremarés de costões rochosos.

**Distribuição Geográfica:** Pantropical.

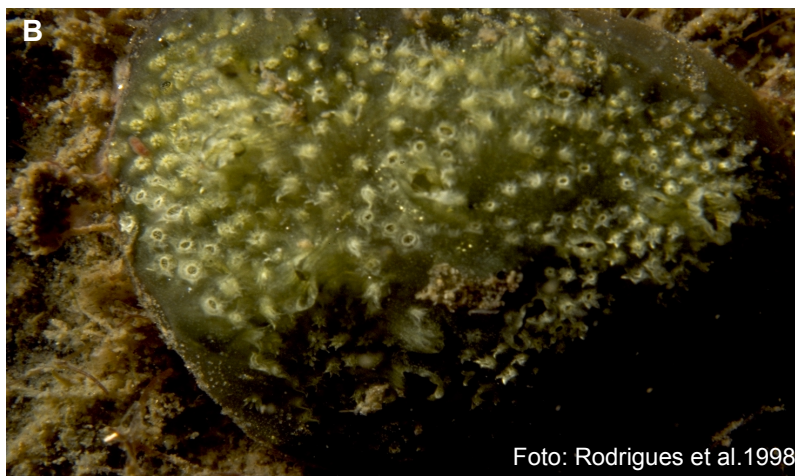
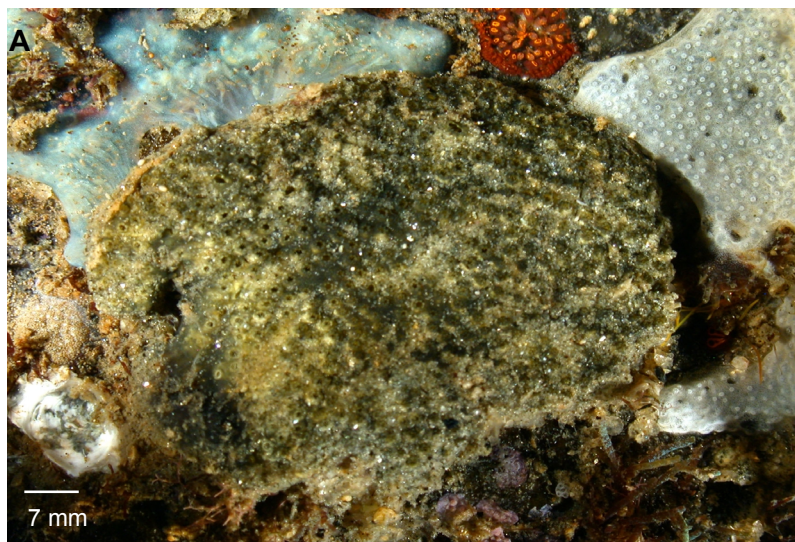


Foto: Rodrigues et al. 1998

## *Aplidium* sp.

(Figs. A-C)

**Tamanho:** Colônias incrustantes com até 1cm de espessura e 15cm de diâmetro. Zoóides alcançando 6,0mm de comprimento.

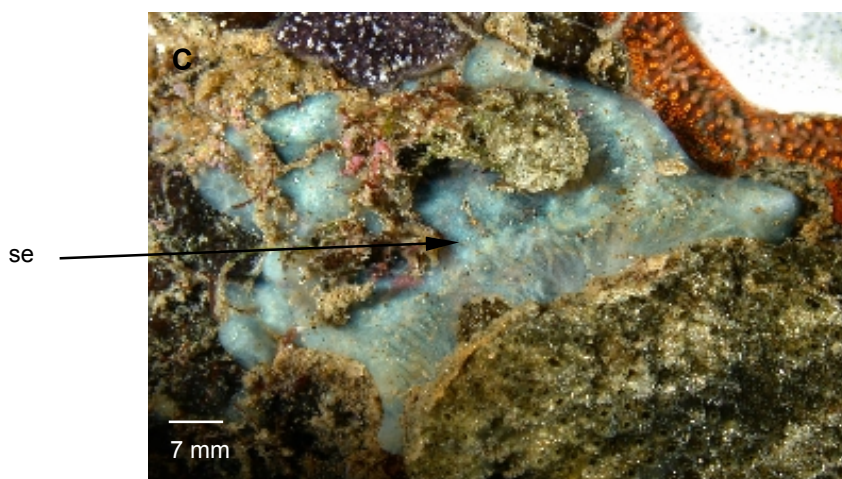
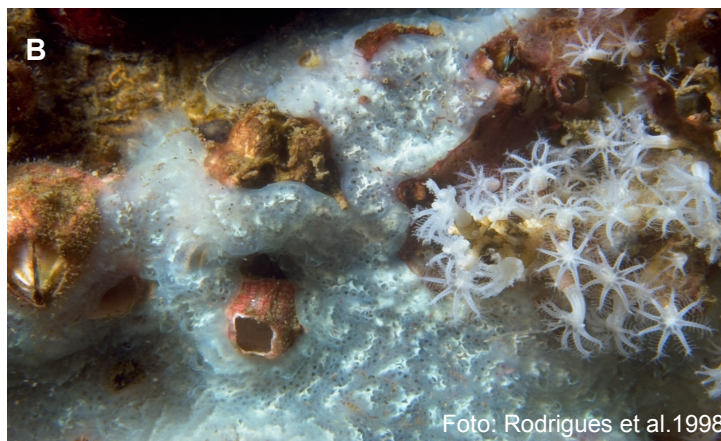
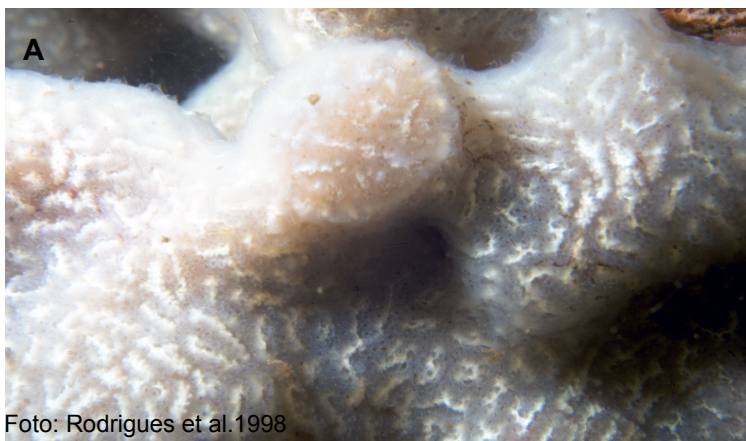
**Identificação:** Colônias de coloração branco leitosa (Fig. A), ou azulada iridescente (Fig. B) em função do reflexo da luz, dando o aspecto característico da espécie. Eventualmente, com algum sedimento (se) na túnica consistente e translúcida (Fig. C).

O zoóide apresenta o sifão branquial com 6 lobos e o atrial formando uma pequena lingüeta. Pós-abdômen continuando do abdômen sem constrição. Estômago alaranjado e com dobras longitudinais. Ovário próximo ao estômago, e testículo na metade terminal do pós-abdômen, com grande número de folículos.

**Comentários:** A coloração iridescente é a característica que permite, em primeira instância, a identificação desta espécie. Além disso, a coloração azulada não é compartilhada com nenhuma outra espécie já registrada para o litoral paulista.

**Habitat:** Desde a região entremarés até infralitoral raso.

**Distribuição Geográfica:** Litoral dos Estados do Rio de Janeiro e São Paulo.



## *Didemnum apersum* Tokioka, 1953

(Figs. A-B)

**Tamanho:** Colônias com 1,5 a 2,0mm de espessura, pequenas, com até 5cm de diâmetro. Zoóides com 0,8 a 1,0mm de comprimento.

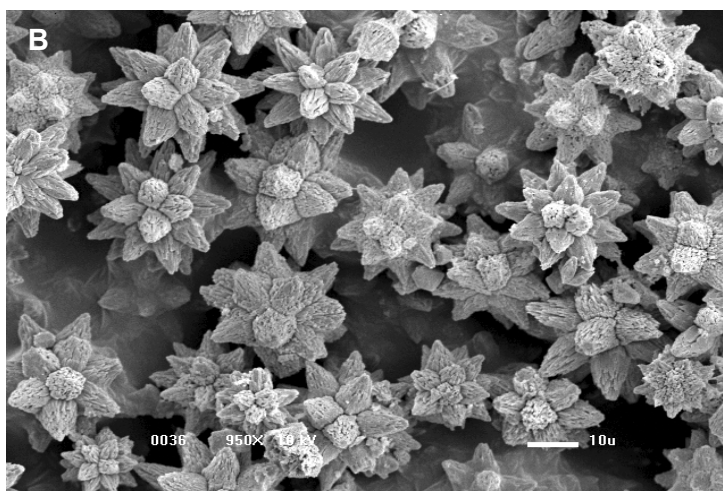
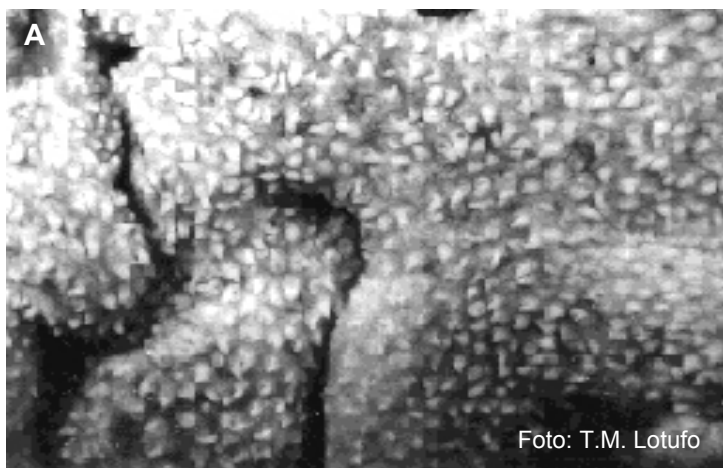
**Identificação:** Colônias brancas, com canais cloacais acinzentados em função da menor quantidade de espículas, dando aspecto marmóreo, assim como em *Didemnum perlucidum*. Distingue-se desta, entretanto, macroscopicamente, pela presença de projeções triangulares (pt), exclusivas da espécie, no bordo de cada sifão branquial (Fig. A). Espículas estreladas com 30 a 50 $\mu$ m de diâmetro (Fig. B).

Zoóide branco, com tórax e abdômen de mesmo tamanho, sifão atrial amplo, expondo parte da cesta branquial. Um único óvulo por zoóide e testículo com um folículo circundado por 6 a 10 voltas do espermiduto.

**Comentários:** São poucas as espécies do gênero *Didemnum* que apresentam alguma forma de projeção na superfície da túnica, sendo esta a característica mais evidente para se ter certeza da identificação de *Didemnum apersum*. Em alguns exemplares as projeções são pouco evidentes a olho nu, sendo porém observadas sob estereomicroscópio.

**Habitat:** Região entremarés de costões rochosos.

**Distribuição Geográfica:** Brasil e Japão.



## *Didemnum cineraceum* (Sluiter, 1908)

(Figs. A-B)

**Tamanho:** Colônias com até 5,0mm de espessura, alcançando 30cm de diâmetro. Zoóides com 1,3 a 1,5mm de comprimento.

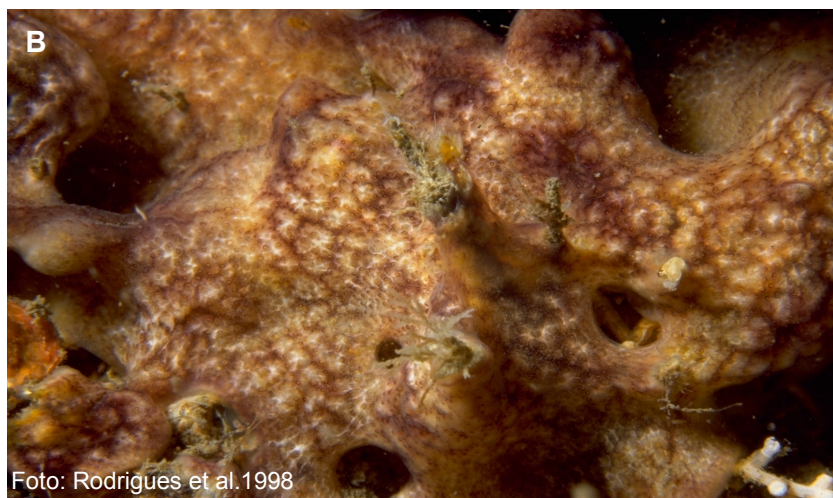
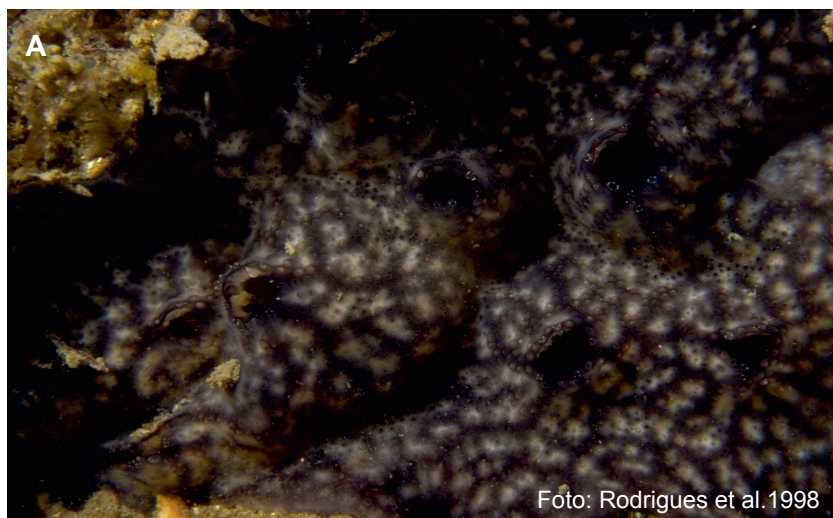
**Identificação:** Colônias de consistência esponjosa e coloração variada: roxo escuro (Fig. A), marrom, castanho (Fig. B) e às vezes com manchas mais claras. Espículas pouco abundantes e com pontas subdivididas.

Zoóide com apêndice fixador tão comprido quanto ou maior que o abdômen. Testículo único, circundado por 6 a 8 voltas do espermiduto. Larva gemípara com até 1,0mm de comprimento, 3 papilas adesivas de pedúnculo longo e 8 ou 9 pares de ampolas.

**Comentários:** As colônias desta espécie podem ser confundidas com as de *Didemnum vanderhorsti* quando em fase inicial de crescimento. É importante atentar para a espessura e consistência da mesma. Para uma confirmação precisa da espécie é de grande interesse a observação de larvas, as quais são inconfundíveis com as de outros "Didemnidae".

**Habitat:** Pouco freqüentes no entremarés, mais abundantes em pequenas profundidades.

**Distribuição Geográfica:** Pantropical.



***Didemnum granulatum* Tokioka, 1954**

(Figs. A-C)

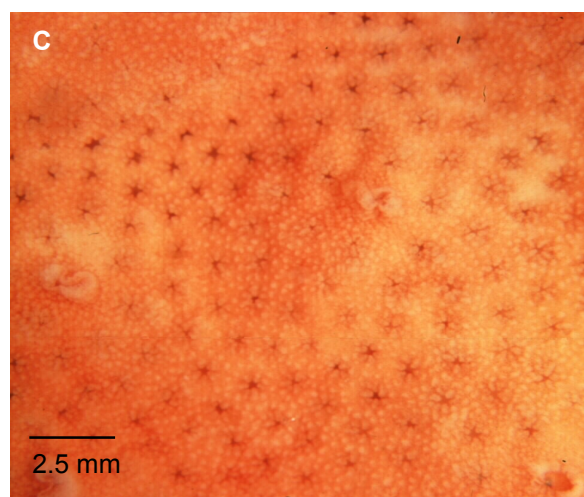
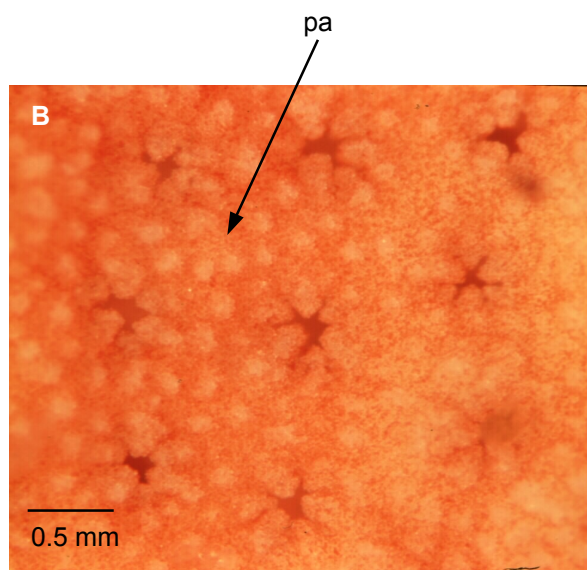
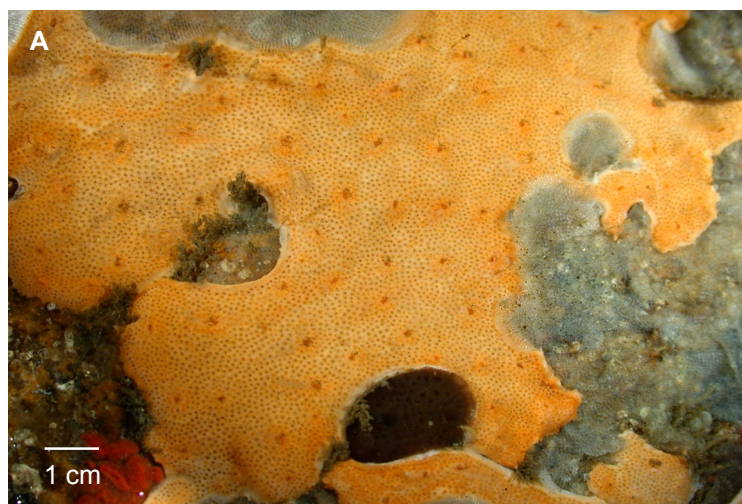
**Tamanho:** Colônias com 2,0mm de espessura, com em média 10 a 15cm de diâmetro, podendo, algumas vezes, alcançar 60cm ou mais. Zoóides medindo em torno de 1,0mm de comprimento, de coloração laranja e de difícil remoção.

**Identificação:** Túnica de coloração laranja ou cor de tijolo, sempre uniforme (Fig. A), repleta de espículas formando pequenas protuberâncias arredondadas (pa), pouco visíveis a olho nu (Fig. B), conferindo um aspecto granulado à superfície da colônia (Fig. C). Larva ovalada com 0,4mm de comprimento, 3 papilas adesivas e 4 pares de ampolas.

**Comentários:** Os pequenos grânulos característicos da espécie e a uniformidade da coloração diferenciam, macroscopicamente, esta espécie de *Didemnum ligulum* e *Didemnum rodriguesi*, as quais, devido à coloração parecida, podem ser eventualmente confundidas.

**Habitat:** Região entremarés de costões rochosos.

**Distribuição Geográfica:** Pantropical.





## *Didemnum ligulum* Monniot F., 1983

(Figs. A-D)

**Tamanho:** Colônias delgadas, 1,0 a 2,0mm de espessura, com até 15cm no seu comprimento maior. Zoóides com 1,5mm de comprimento.

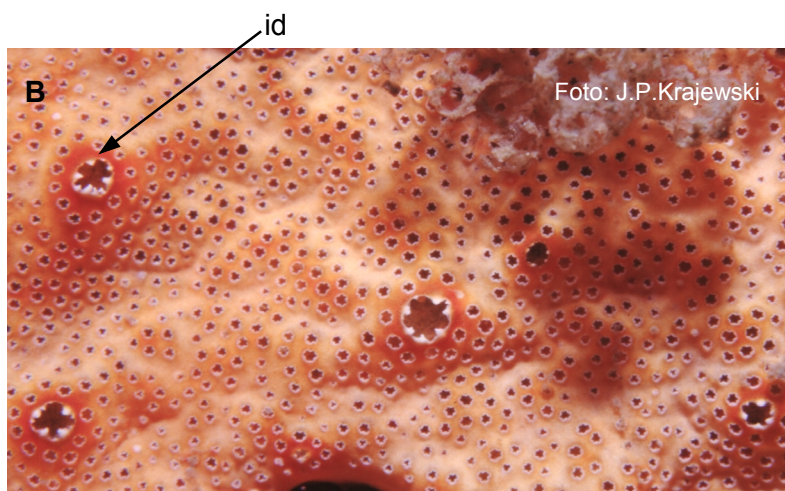
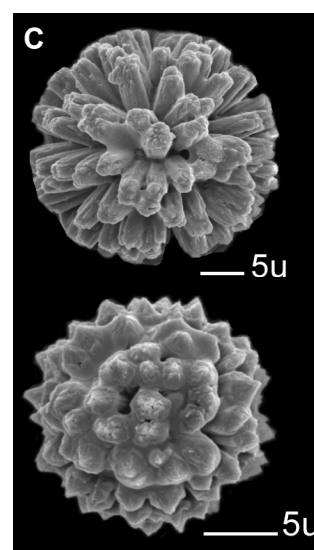
**Identificação:** Colônias de cor alaranjada ou amarelada, não uniforme (Fig. A). Coloração mais intensa próximo às cloacas, as quais apresentam a borda rodeada internamente por identações de cor branca (id) (Fig. B). Cloacas e sífões inalantes circundados por muitas espículas o que lhes conferem uma aparência estrelada.

Zoóide alaranjado com sífão branquial tubular com 6 longos lobos, cavidade atrial ampla com uma pequena lingüeta. abdômen com pigmento concentrado na região das gônadas. O testículo apresenta um único lobo, circundado parcialmente por 7 voltas anti-horárias do espermiduto. Ovário com um óvulo. Larva laranja, gemípara, com três pares de papilas adesivas e 10 ou mais pares de ampolas. Espículas com raios curtos pontiagudos ou compridos e rombudos, com 10 a 50 $\mu$ m de diâmetro (Fig. C).

**Comentários:** O padrão de coloração não-uniforme é o primeiro indicativo desta espécie. Uma observação detalhada das identações na borda das cloacas permite a identificação da espécie a olho nu. O número de ampolas na larva também é caráter que distingue *D. ligulum* das restantes. Quando pequenas (Fig. D), a identações podem ser pouco visíveis assim como o padrão não-uniforme da coloração, sendo necessárias as observações dos zoóides, larvas e espículas.

**Habitat:** Desde a região entremarés até pequenas profundidades.

**Distribuição Geográfica:** Atlântico tropical americano e Polinésia.



## *Didemnum lutarium* Van Name, 1910

(Fig. A)

**Tamanho:** Colônias com 2,0mm de espessura e até 5cm de comprimento no seu maior diâmetro. Zoóides com até 1,3mm de comprimento.

**Identificação:** Colônias com túnica branca leitosa ou ligeiramente bege, geralmente lisa e brilhante (Fig. A). Eventualmente, devido à ausência de espículas na camada superficial da túnica, pode-se observar o contorno dos zoóides. As espículas são arredondadas e muito abundantes nas regiões inferiores da túnica.

O zoóide é róseo e facilmente removido da túnica. Apresenta sifão branquial longo e abertura atrial pequena. Ambos os sexos são encontrados no mesmo zoóide, sendo o ovário observado com um óvulo grande e diversos menores em desenvolvimento. Já o testículo apresenta dois lobos circundados por 6 a 9 voltas do espermiduto. A larva apresenta 3 papilas adesivas e 4 pares de ampolas.

**Comentários:** Esta espécie é a única com testículo bilobado com registro para o litoral paulista, o que torna fácil a sua identificação a partir dos zoóides.

**Habitat:** Região entremarés de costões rochosos.

**Distribuição Geográfica:** Atlântico tropical americano, desde o Golfo do México até o norte dos Estados Unidos.



***Didemnum perlucidum* Monniot F., 1983**  
(Figs. A-C)

**Tamanho:** Colônias delgadas (1,0mm), podendo atingir 40cm de comprimento. Zoóides medindo em torno de 1,0mm de comprimento.

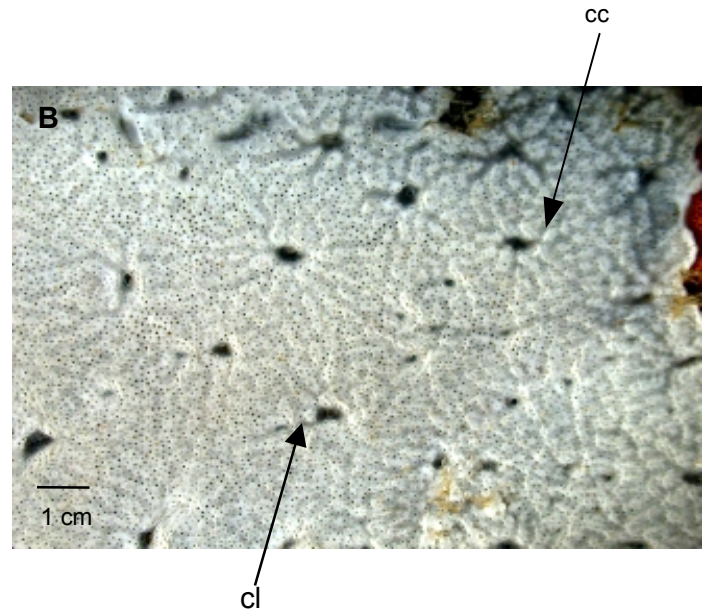
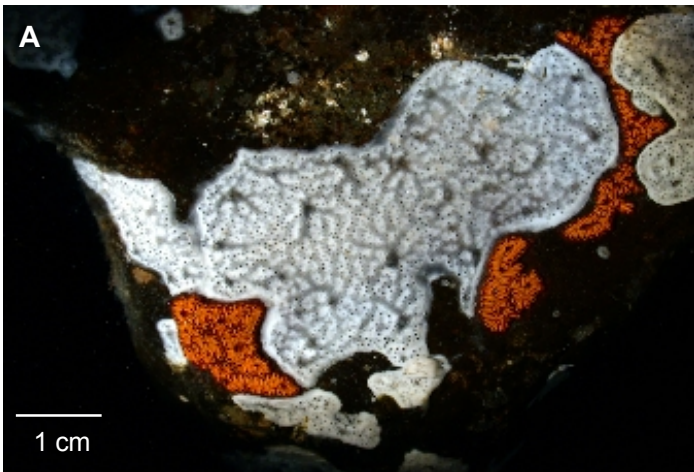
**Identificação:** Colônias incrustantes, de coloração branco gelo com os canais cloacais(cc) de coloração acinzentada em função da menor abundância de espículas nessa região (Fig. A). Inúmeras cloacas (cl) grandes e redondas (Fig. B).

Zoóide branco com estômago alaranjado, testículo único com 5 a 7 voltas do espermiduto, ovário com um óvulo grande e outros pequenos. Larva, arredondada, com menos de 0,5mm de diâmetro, com 3 papilas adesivas e 4 pares de longas ampolas.

**Comentários:** Esta espécie é muito comum em todo litoral paulista, podendo dominar grandes área de costão. Eventualmente é observada formando projeções em forma de gotas quando crescendo em face inferior de superfícies inclinadas (Fig. C).

**Habitat:** Encontrada desde a região entremarés até 5m de profundidade.

**Distribuição Geográfica:** Pantropical.



*Didemnum psammotodes* (Sluiter, 1895)

(Figs. A-C)

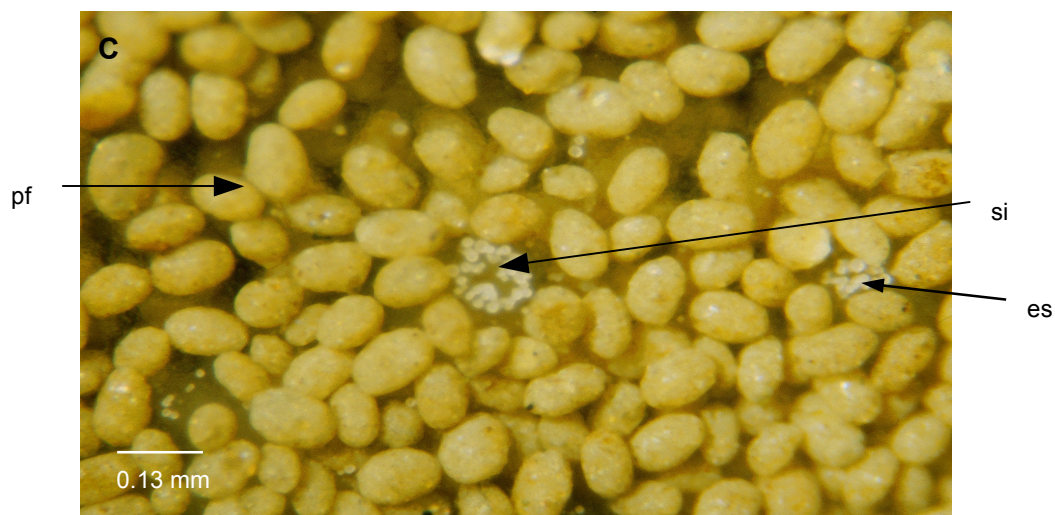
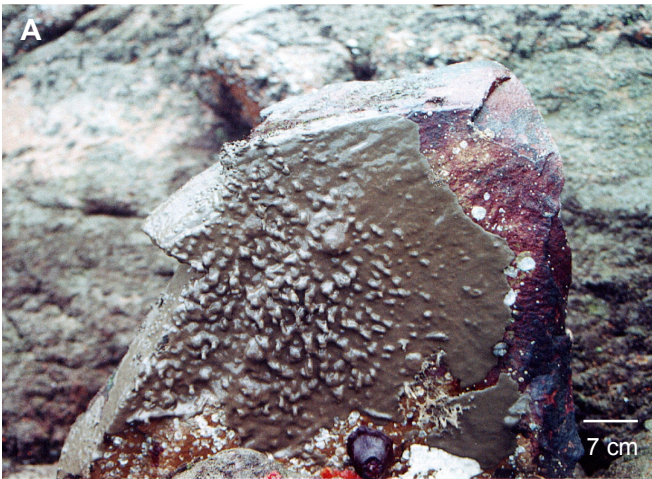
**Tamanho:** Colônias incrustantes, delgadas, com 1,0 ou 2,0mm de espessura, podendo formar grandes placas de 20cm ou mais de diâmetro. Zoóides pequenos medindo de 0,4 a 1,0mm de comprimento.

**Identificação:** Colônias de coloração parda ou marrom, lembrando sedimento arenoso ou lodoso (Figs. A-B), devido ao intenso acúmulo de pelotas fecais (pf) na superfície da colônia, o que impossibilita a visualização dos zoóides (Fig. C). Cloacas presentes em grande número por toda colônia. Espículas (es) quase esféricas com numerosos raios de pontas irregulares abundantes, restritas ao redor dos sífones inalantes (si) dos zoóides.

**Comentários:** Esta é a única espécie da Família Didemnidae de coloração marrom no litoral paulista. É geralmente a espécie de ascídia colonial que ocorre nas regiões mais superiores do costão.

**Habitat:** Região entremarés de costões rochosos.

**Distribuição Geográfica:** Pantropical. No Brasil já foi encontrada em praticamente todo o litoral.



## *Didemnum speciosum* (Herdman, 1886)

(Figs. A-C)

**Tamanho:** Colônias delgadas (1,0 a 2,0mm), com 1 a 4cm de comprimento. Zoóides com menos de 1,0mm de comprimento.

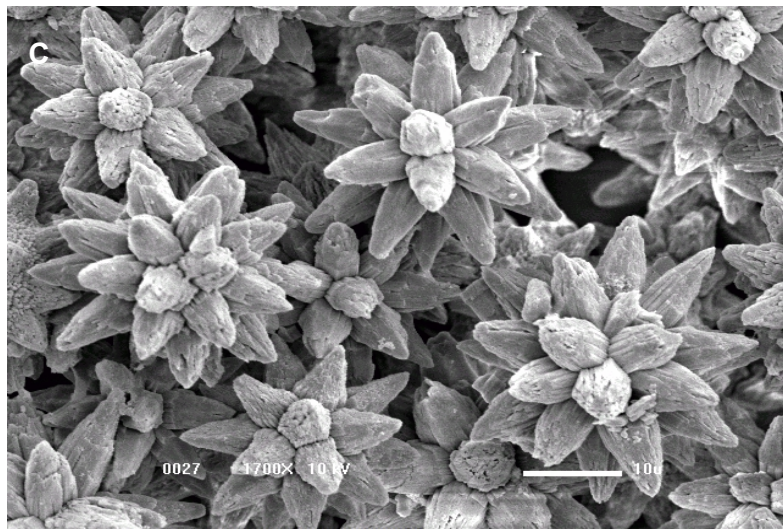
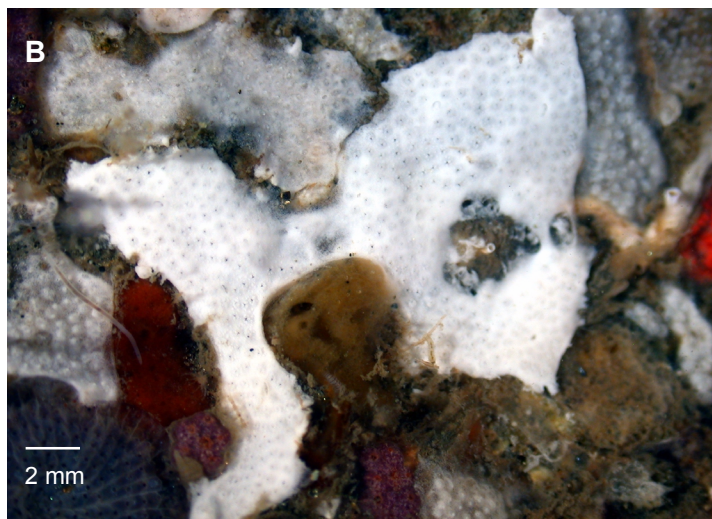
**Identificação:** Colônias de coloração branca, muito comuns sobre algas talosas e substratos artificiais (Figs. A-B). Apresenta túnica quebradiça, em função da grande concentração de espículas estreladas com raios cônicos longos e pontiagudos (Fig. C).

Zoóide incolor, sifão atrial amplo, expondo parte da cesta branquial. Ovário com um óvulo maduro e outros menores. Testículo único, circundado por 6 voltas anti-horárias do espermiduto. Larva com 0,4 a 0,6mm de comprimento, com 4 ampolas e 3 papilas adesivas.

**Comentários:** Dentre os didemnídeos brancos é a espécie mais comum sobre talos de algas. É provavelmente a primeira espécie descrita originalmente para o Brasil.

**Habitat:** Desde a região entremarés até pequenas profundidades. Frequentes em substratos artificiais e talos de macroalgas.

**Distribuição Geográfica:** Litoral brasileiro, de São Sebastião ao Ceará.



*Didemnum vanderhorsti* Van Name, 1924

(Figs. A-B)

**Tamanho:** Colônias incrustantes, delgadas com 1,0 a 5,0mm de espessura, raramente ultrapassando 20cm de diâmetro. Zoóides pequenos com até 1,0mm de comprimento.

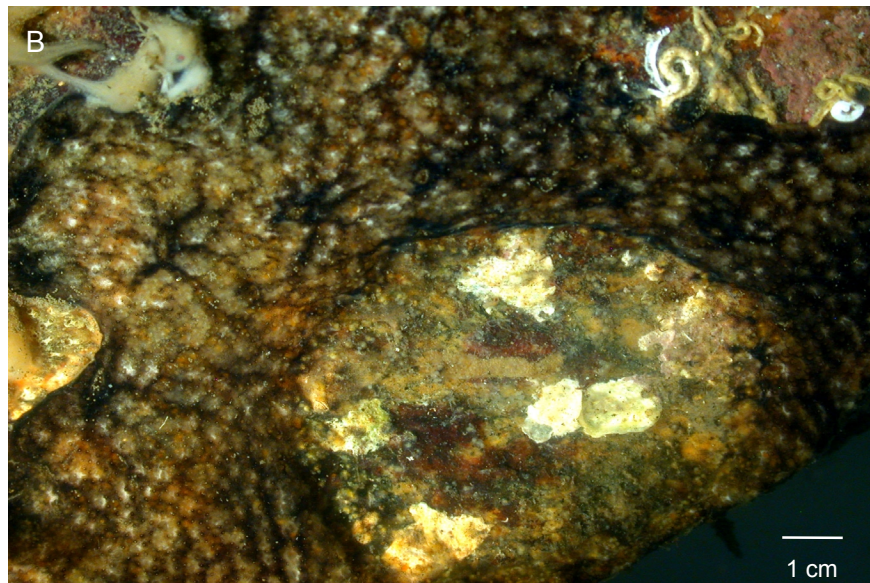
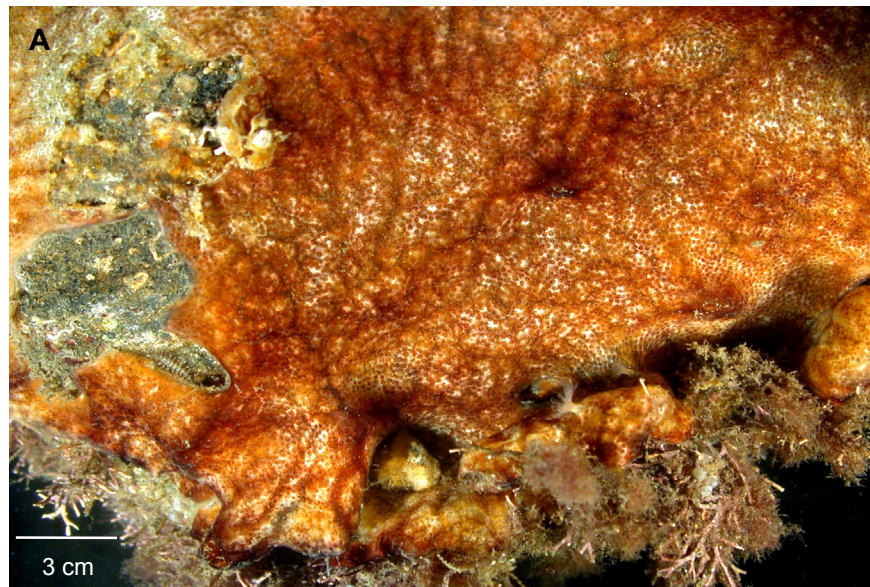
**Identificação:** Colônias de coloração castanha, roxo escuro ou marrom (Fig. B), às vezes mais clara (Fig. A). Túnica lisa, de consistência firme, apresentando espículas pouco abundantes, pequenas (10 a 35 µm), esféricas com numerosos raios curtos, ausentes em alguns espécimes.

Larva medindo cerca de 0,5mm, com 3 papilas adesivas e 4 pares de ampolas. Cauda circundando  $\frac{3}{4}$  do tronco.

**Comentários:** Esta espécie é uma das mais abundantes em regiões rasas do costão rochoso, chamando atenção pela variação cromática das colônias.

**Habitat:** Região entremarés, crescendo em locais sombreados e sob rochas.

**Distribuição Geográfica:** Atlântico tropical americano.



## *Didemnum* sp.1

(Figs. A-B)

**Aspecto externo:** Colônias brancas, beges ou amareladas, de aspecto não uniforme, delgadas (<2,0mm), raramente ultrapassando 10cm no seu comprimento maior, encontradas nas regiões superiores do entremarés, sob rochas. Cloacas evidentes formando princípios de canais cloacais, porém nunca formando uma malha de canais como em *Didemnum perlucidum*. Espículas de duas formas principais, estreladas com numerosos raios curtos e extremidades rombudas ou com poucos raios cônicos e extremidades arredondadas. Ambas as formas com aproximadamente 30µm de diâmetro (Fig. A), pouco abundantes, concentradas próximo a região do sifão branquial, na camada superficial da túnica.

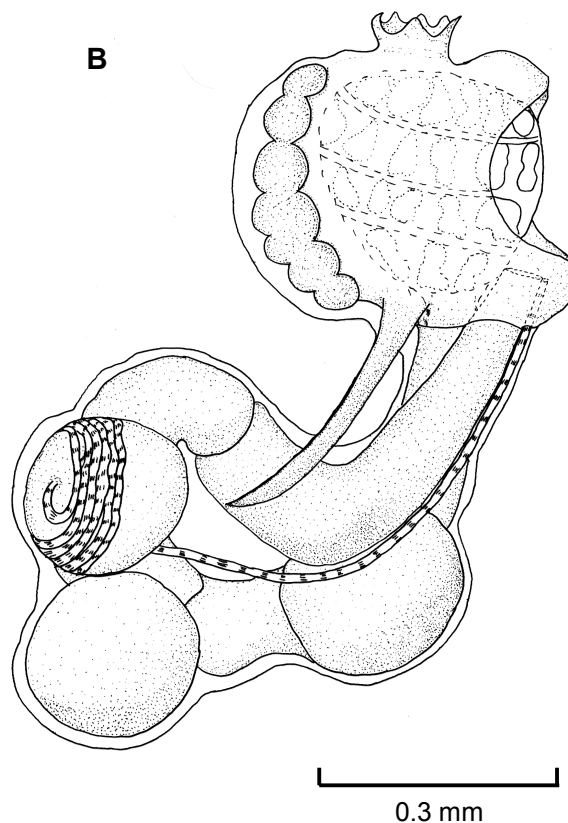
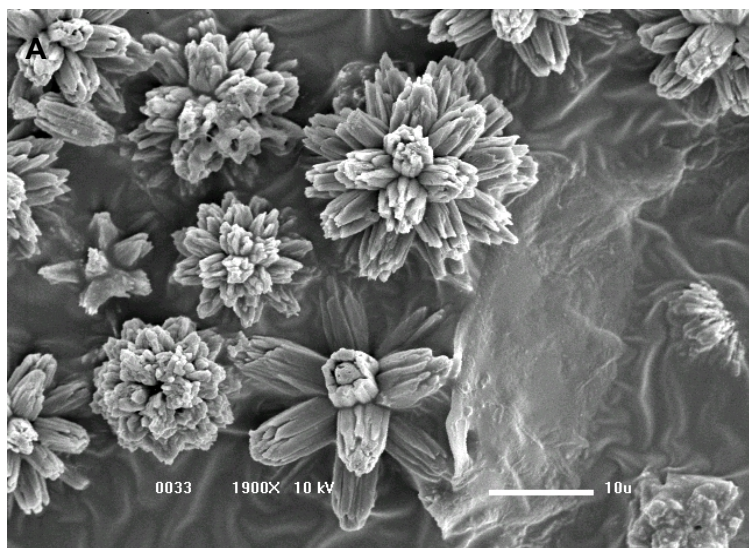
**Zoóides:** pequenos, medindo menos que 1,0mm, translúcidos, apresentando sifão branquial com seis lobos e atrial em forma de fenda, expondo parte da cesta branquial. Esta é típica do gênero, apresentando 4 fileiras de fendas com 6 fendas na primeira e 3 na última fileira. Apêndice fixador longo com o dobro do tamanho do tórax.

Abdômen típico do gênero, estômago globular, trato digestório com duas constrictões. Ovário sobre a alça intestinal com um óvulo grande e eventualmente 2 pequenos abaixo do testículo indiviso, circundado parcialmente por 6 voltas do espermiduto (Fig. B).

**Larvas:** Larvas com 0,6mm de comprimento, 4 pares de compridas ampolas e 3 papilas adesivas também longas. Cauda circundando todo o corpo da larva.

**Comentários:** A descrição destes exemplares é muito semelhante à de *Didemnum ahu*. No entanto, diferentemente desta espécie, foram encontradas colônias consideravelmente grandes e geralmente de coloração branca. Além disso, apesar da presença dos dois tipos de espículas descritos para *D. ahu*, o tipo com maior número de raios não os apresentou com formato cônico, como descrito originalmente. Sem mais exceções, os exemplares coletados se enquadram na espécie *Didemnum ahu*.

**Distribuição Geográfica:** Praia da Baleia e Praia Grande, São Sebastião-SP.



***Didemnum* sp.2**

(Figs. A-B)

**Aspecto externo:** Colônias brancas com até 10cm de diâmetro, devido ao acúmulo de espículas, principalmente sobre os sífões branquiais. Espículas pouco abundantes, com muitos raios rombudos e curtos, nunca cilíndricos como em *Didemnum ahu*.

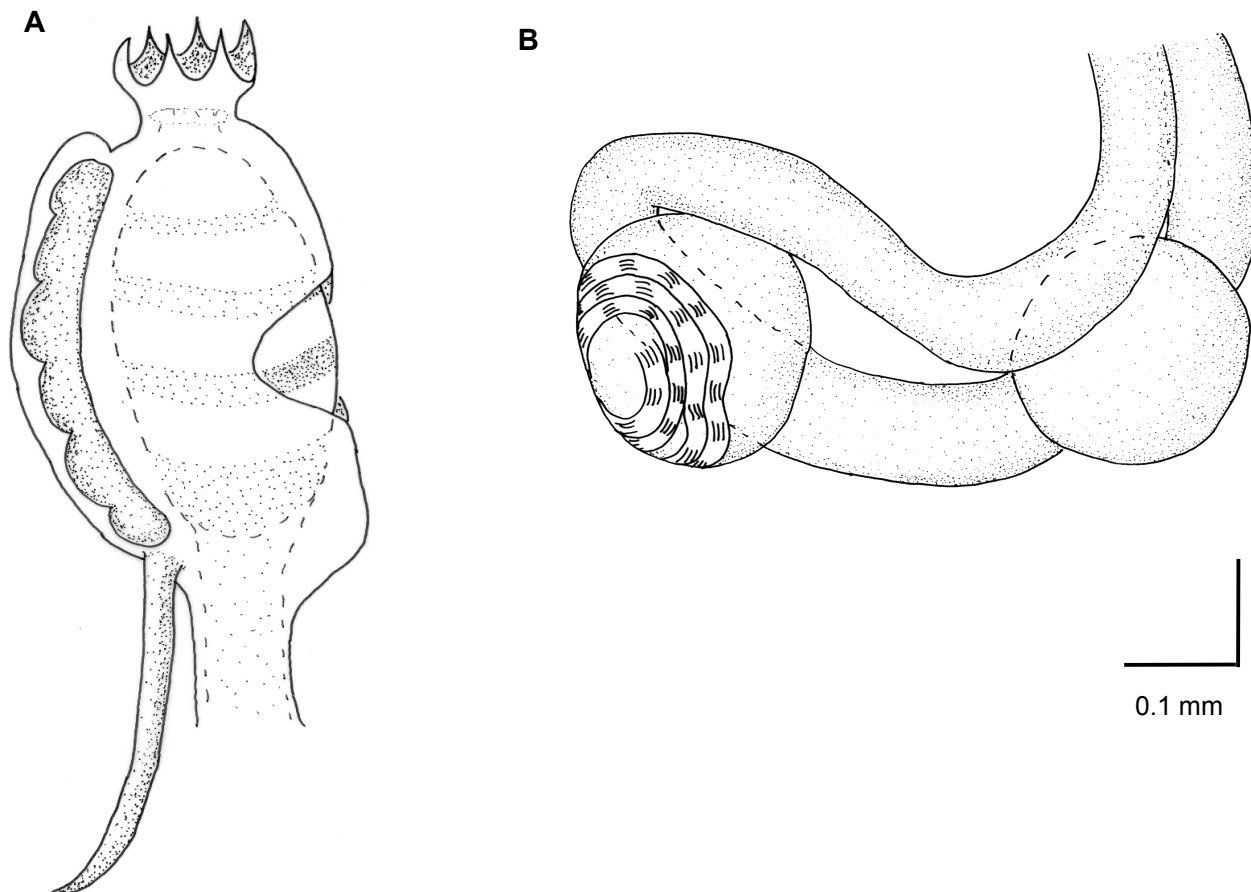
**Zoóides:** com 1,0mm de comprimento, brancos ou levemente rosados, apresentando sífão branquial com 6 lobos longos e atrial em forma de uma pequena fenda, entre a segunda e terceira fileira de fendas branquiais. Tórax (Fig. A) duas vezes mais comprido do que largo, com um apêndice fixador com  $\frac{3}{4}$  do tamanho do tórax.

Abdômen (Fig. B) largo com trato digestório delgado apresentando 2 alças intestinais características do gênero. Estômago redondo e pouco evidente, de coloração branca. Testículo único, parcialmente coberto por 4 voltas do espermiduto, sob a alça intestinal. Ovário não observado.

**Larvas:** Não observadas.

**Comentários:** Apesar de se assemelhar muito com *Didemnum* sp1, pode ser facilmente distinguível, a olho nu, pela menor densidade de espículas. Além disso o abdômen é muito mais comprido que o de *Didemnum* sp1, apresentando ainda uma menor fenda atrial. A identificação da espécie ficou comprometida devido à não observação de larvas e ovário.

**Distribuição Geográfica:** Praia da Baleia.





### *Didemnum* sp.3

(Figs. A-C)

**Aspecto externo:** Colônias pequenas nunca maiores que 5cm de comprimento. Encontradas duas vezes no entremarés da Praia da Baleia, São Sebastião-SP. Túnica repleta de espículas com raios cônicos curtos na camada superficial, principalmente ao redor dos zoóides, não permitindo a visualização destes na colônia intacta. Nas camadas inferiores, entretanto, as espículas são pouco abundantes.

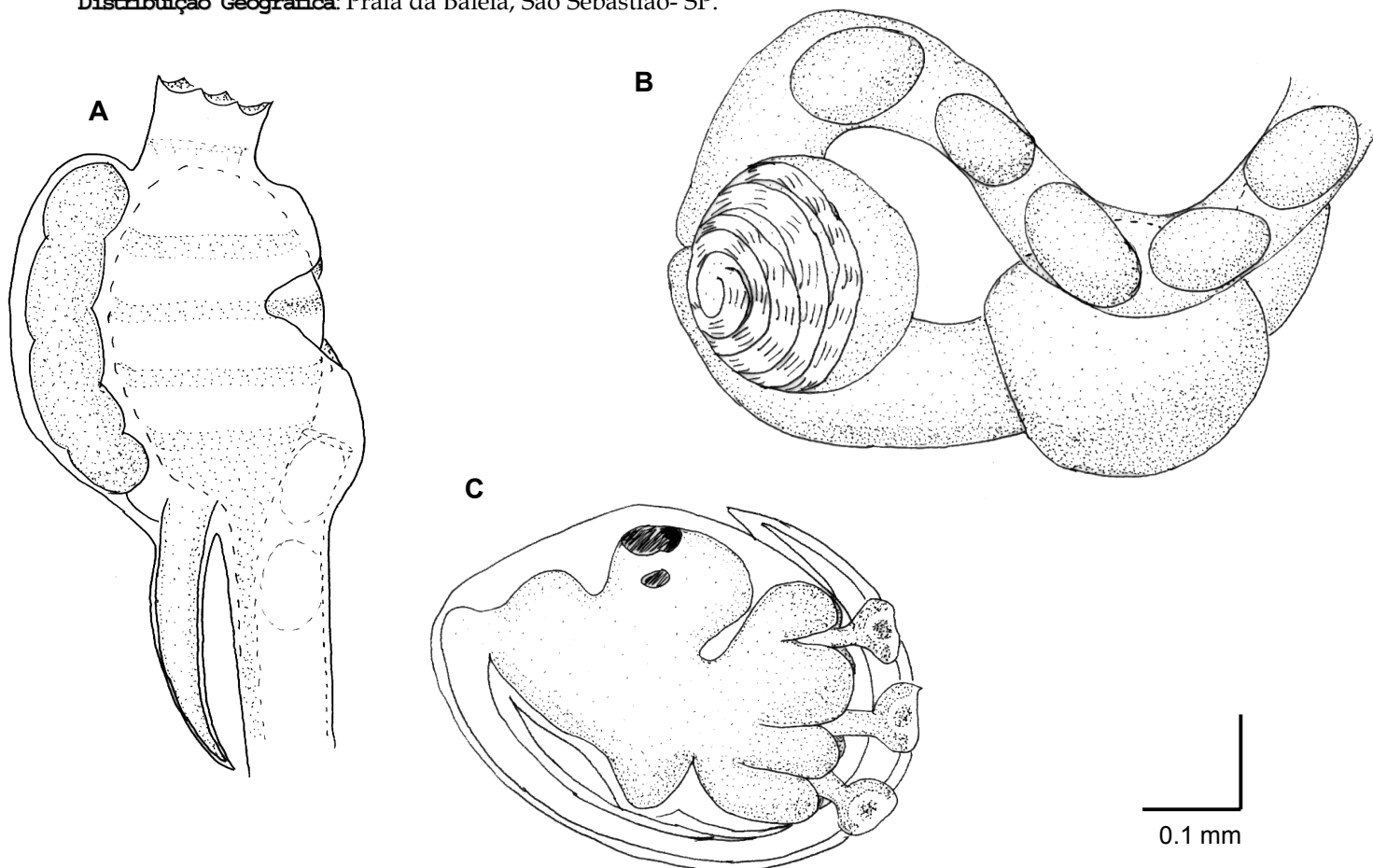
**Zoóides:** com aproximadamente 1,0mm de comprimento, removidos com dificuldade da túnica. Pedúnculo esofágico-retal envolto por uma camada de espículas, eventualmente estendendo-se até a porção mediana do tórax, o qual apresenta coloração alaranjada que desbota após a fixação (Fig. A). Sifão branquial comprido e amplo com 6 lobos afilados e pouco definido em alguns zoóides. Cesta branquial com 4 fileiras de fendas e sifão atrial em forma de pequena abertura expondo parte da cesta branquial na altura da segunda fileira. Exceto pelos zoóides em formação, todos os abdômens apresentam uma pelota fecal próxima ao ânus. Apêndice fixador de tamanho igual ou um pouco menor que o tórax.

Abdômen (Fig. B) com duas dobras intestinais bem marcadas, apresentando uma constrição antes da primeira. Estômago circular de coloração igual a todo o resto do intestino. Ovário com um óvulo aderido internamente à alça intestinal. Testículo no mesmo local com um lobo e espermiduto em espiral (5 voltas). Zoóides sempre com inúmeras pelotas fecais acumuladas no trato digestivo, nunca apresentando ambas as gônadas simultaneamente.

**Larvas:** pequenas (0,5mm) com quatro pares de ampolas e três papilas adesivas. Cauda circundando  $\frac{3}{4}$  do corpo da larva (Fig. C).

**Comentários:** Os dois exemplares coletados não diferem externamente dos descritos como *Didemnum* sp2, porém a análise dos zoóides permitiu a observação de inúmeras diferenças. A análise de um número maior de colônias se faz necessário para a identificação da espécie.

**Distribuição Geográfica:** Praia da Baleia, São Sebastião- SP.



## *Didemnum* sp.4

(Figs. A-F)

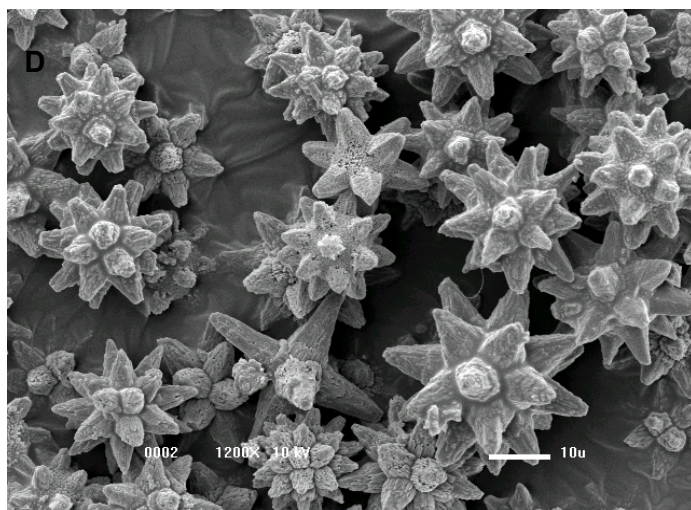
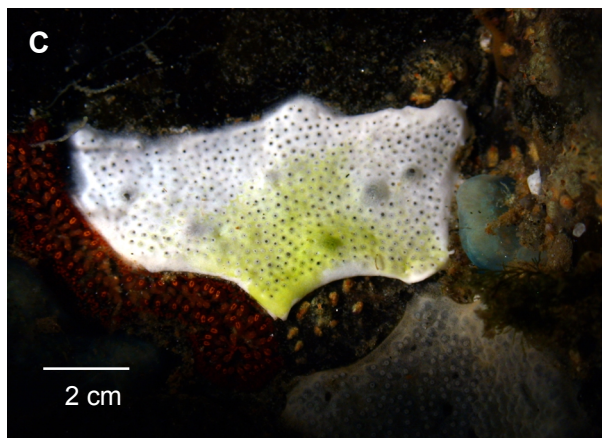
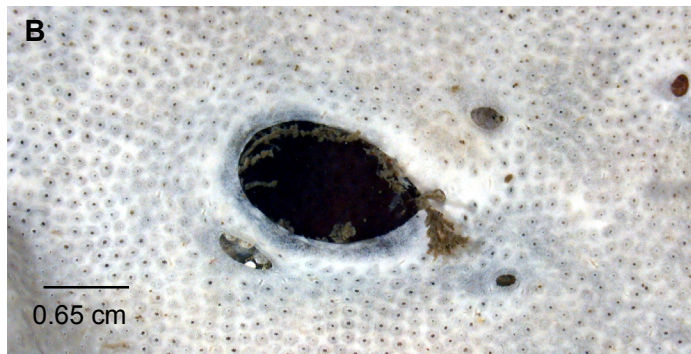
**Aspecto externo:** Colônias espessas (1,5mm) com até 10cm de diâmetro, de coloração branco gelo, as vezes com manchas verdes de algas simbiotes (Figs A-C). Túnica de consistência macia, repleta de espículas estreladas cônicas de extremidades truncadas, com 20 a 30µm de diâmetro localizadas na camada superficial (Fig. 4) e restritas ao redor dos zoóides nas camadas inferiores da túnica. Cloacas grandes e abundantes. São pouco abundantes em todo entremarés, sendo encontradas principalmente durante os meses mais quentes, na franja do infralitoral.

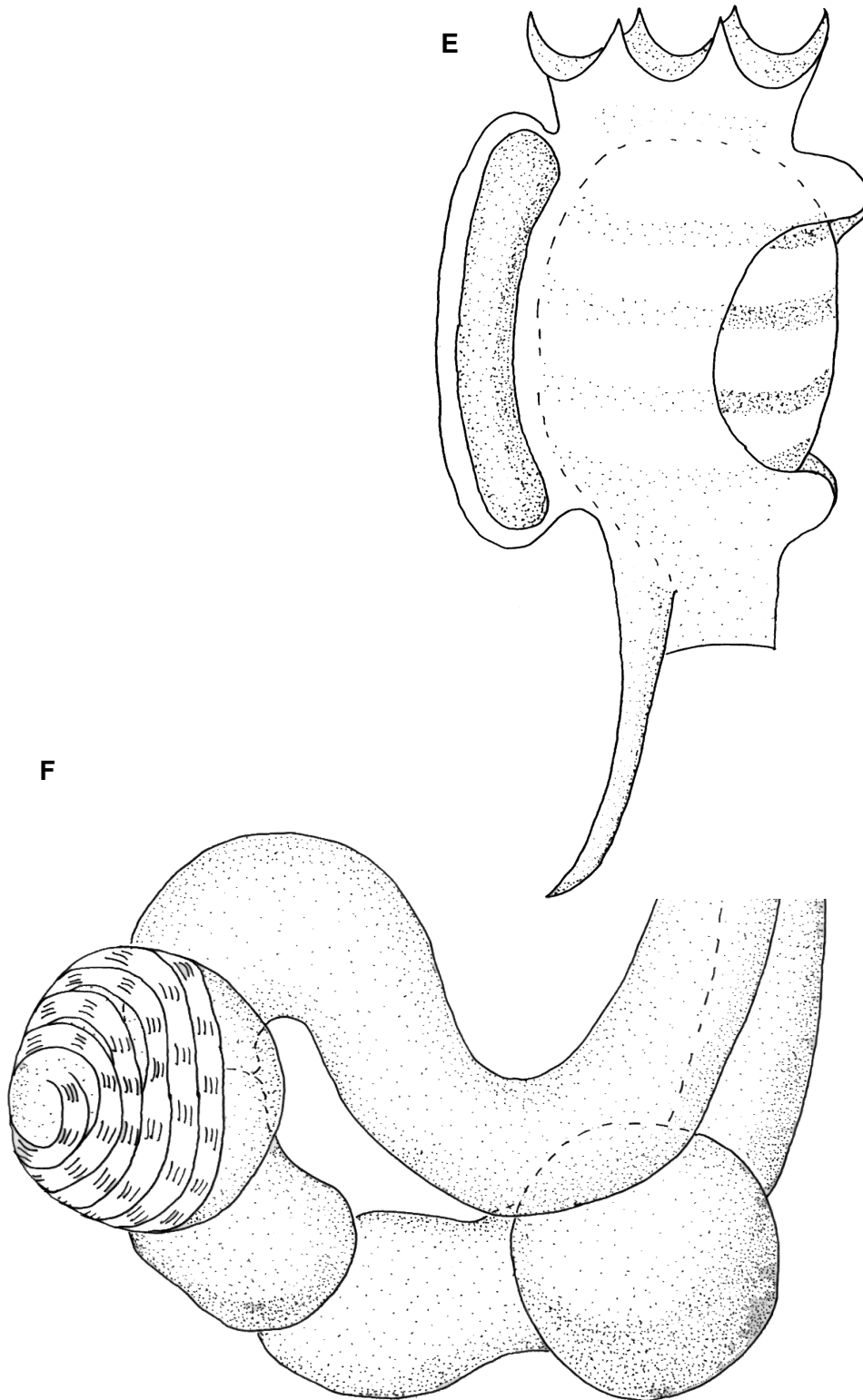
**Zoóides:** pequenos, com menos de 1,0mm de comprimento, facilmente removíveis, envoltos por uma fina camada de túnica repleta de espículas, o que dificulta a observação dos mesmos. Sifão branquial curto e muito amplo com 6 lobos proeminentes. Sifão atrial em forma de fenda muito ampla expondo grande parte das 4 fileiras de fendas branquiais. Pedúnculo esofágico com metade do tamanho do tórax (Fig. 5). Trato digestório com dobras características e bem marcadas do gênero, com duas constrições no intestino, uma antes e outra no meio da primeira alça intestinal, a segunda recobrimdo parcialmente o estômago, o qual é ovalado e esbranquiçado como todo o trato digestório. Testículo único localizado sobre a alça intestinal, envolto por 4 a 6 espirais horárias do espermiduto (Fig. 6). Gônadas femininas não observadas.

**Larvas:** Não observadas.

**Comentários:** Não há no litoral brasileiro nenhuma espécie de *Didemnum* com espículas de extremidade truncada e nem com o padrão de distribuição de espículas conforme observado nos exemplares de *Didemnum* sp6. Tal fato pode indicar uma nova ocorrência, ou mesmo a eleição de uma nova espécie. A observação de ovário e larvas é necessária para melhor definição da espécie.

**Distribuição Geográfica:** Canal de São Sebastião no Estado de São Paulo.





0.1 mm

## *Diplosoma macdonaldi* Herdman, 1841

(Figs. A-C)

**Tamanho:** Colônias delgadas (2,0mm), em média, com 10cm de diâmetro, podendo, no entanto, alcançar até 20cm no seu maior comprimento. Zoóides com 1,0mm de comprimento.

**Identificação:** Colônias transparentes, sem espículas, delicadas e de difícil remoção da rocha em função de sua frágil consistência (Fig. A). Pigmento branco (pb) presente na túnica, delimitando os sífões branquiais (Fig. B).

Zoóide apresenta o abdômen (ab) pigmentado de marrom escuro, visível através da túnica (Fig C). Sifão branquial e atrial muito amplos. Estômago globular de coloração laranja. Testículo bilobado situado sob a alça intestinal com espermiduto reto, sem espirais. Ovário com um óvulo maduro e até 4 pequenos. Larva gemípara, com 3 papilas adesivas e 4 pares de ampolas.

**Comentários:** Esta espécie é facilmente reconhecida em função da sua fragilidade e da ausência de espículas. O baixo investimento na produção da túnica permite um crescimento rápido das colônias, as quais são mais freqüentes e maiores nos meses mais quentes de verão e raras e pequenas nos meses de inverno.

**Habitat:** Região entremarés de costões rochosos.

**Distribuição Geográfica:** Atlântico tropical americano.



## *Trididemnum orbiculatum* (Van Name, 1902)

(Fig. A-D)

**Tamanho:** Colônias incrustantes com até 30cm de diâmetro. Espessas (1cm) no infralitoral e delgadas (2,0 a 3,0mm) no entremarés. Zoóides raramente ultrapassando 1,0mm de comprimento.

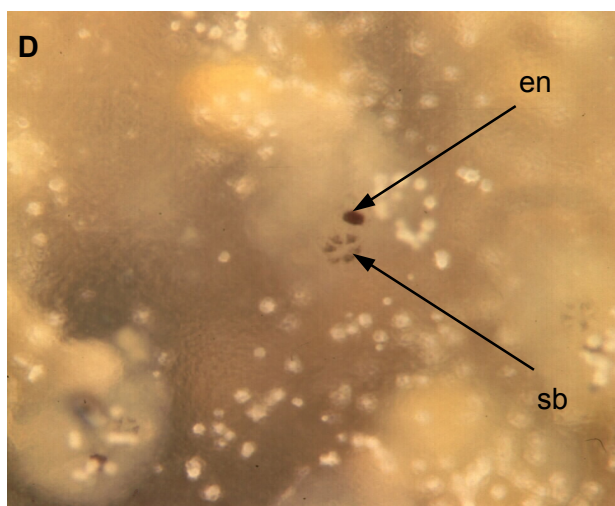
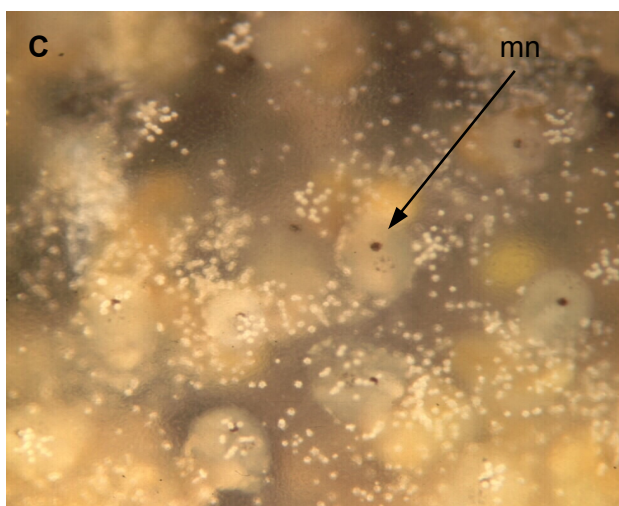
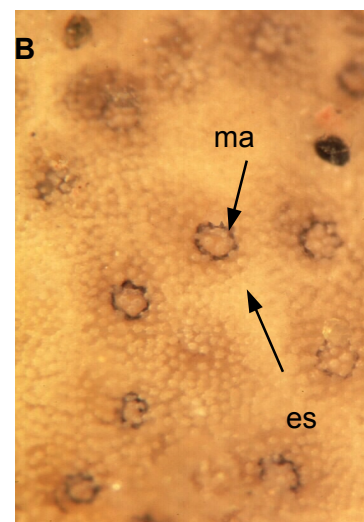
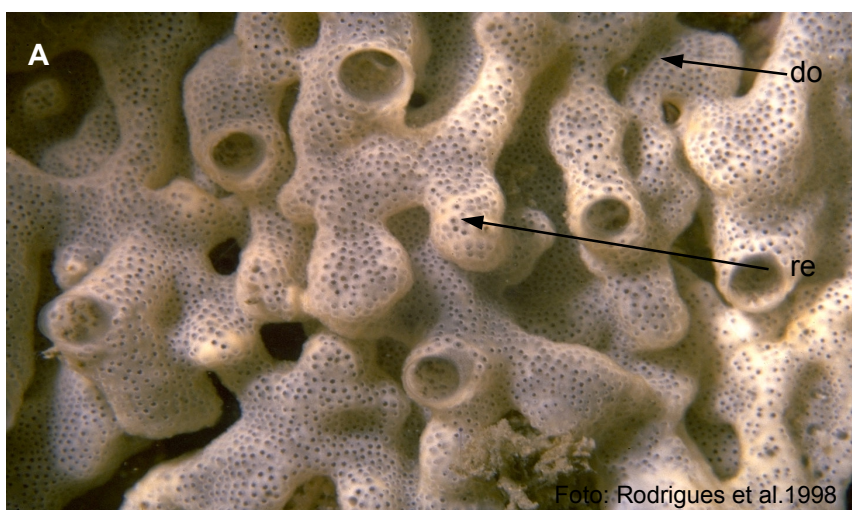
**Identificação:** Colônias no infralitoral de coloração branco-acinzentada, devido a combinação da coloração escura do manto (ma) dos zoóides com a clara das espículas (es) (Fig A). Eventualmente pode se observar dobras (do) e reentrâncias (re) na túnica dando um aspecto de vela derretida às colônias. As espículas, abundantes na camada superior da túnica, são grandes e estreladas. No entremarés as colônias têm a coloração esverdeada (Fig. C), amarelada ou acinzentada e baixa concentração de espículas, o que permite a visualização dos zoóides. Estes apresentam uma mancha negra (mn) na região anterior do endóstilo (en) e as extremidades dos lobos do sífão branquial (sb) também pigmentadas (Fig D).

Zoóide com abertura atrial tubular, característica do gênero. Testículo único, circundado por 5 a 7 voltas do espermiduto. Larva pequena com 4 pares de ampola e 3 papilas adesivas.

**Habitat:** Desde o entremarés até o infralitoral, geralmente em face inferior e lateral de rochas ou crescendo sobre o talo de macroalgas.

**Comentários:** O dimorfismo, acima citado, é motivo de muita discussão. Atualmente alguns autores propõem a separação desses morfotipos em duas espécies, em função das enormes diferenças, tanto morfológicas como ecológicas.

**Distribuição Geográfica:** Atlântico tropical americano.



*Trididemnum* sp.

(Figs. A-B)

**Aspecto externo:** Colônia com 2cm em seu maior comprimento, de coloração bege, coletada no entremarés da praia da Baleia, São Sebastião-SP. Túnica repleta de espículas grandes de raios pontiagudos como em *Trididemnum orbiculatum*, diferenciando-se, entretanto, pela formação de projeções em forma de torre devido ao acúmulo de inúmeras camadas sobrepostas de espículas.

**Zoóides:** pequenos, com menos de 1,0mm, de coloração rósea, com tórax mais largo que comprido (Fig. A). Sifão branquial amplo e com seis lobos discretos e o atrial amplo, tubular com bordo liso projetado em direção anterior do tórax. Cesta branquial com três fileiras de fendas totalmente imersas no tórax, apêndice fixador muito reduzido ou ausente.

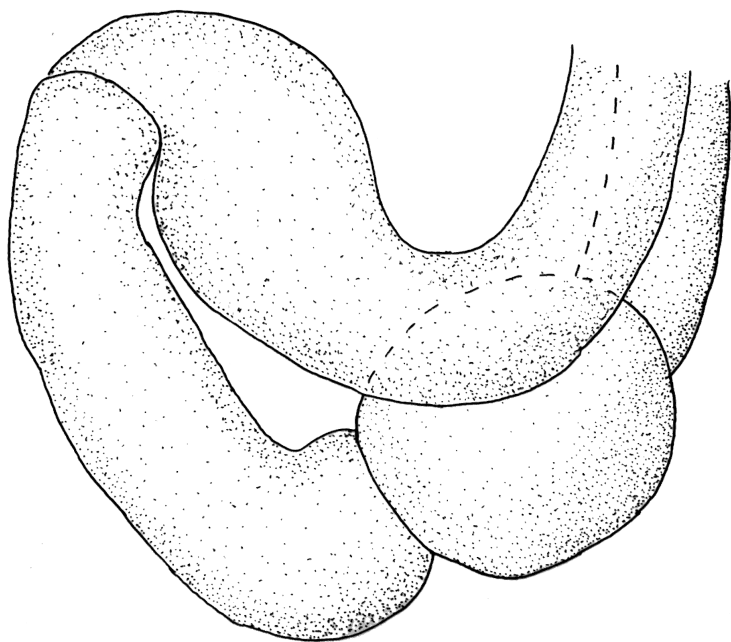
Abdômen com duas alças intestinais e uma constrição. Estômago ovalado e parcialmente recoberto pela segunda alça (Fig. B). Gônadas não observadas.

**Larvas:** Não observadas.

**Comentários:** A separação desta única colônia como uma espécie diferente de *Trididemnum orbiculatum* se deve às projeções na superfície da túnica, as quais são pouco freqüentes na família. Porém, devido à ausência de gônadas e larvas, é necessário a obtenção de novas colônias para maiores afirmações.

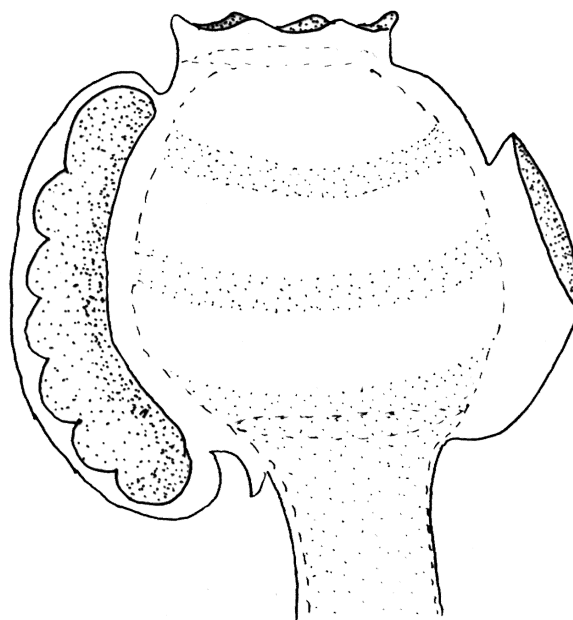
**Distribuição Geográfica:** Praia da Baleia, São Sebastião-SP.

A



0.19 mm

B



0.12 mm

*Polysyncraton amethystrum* (Van Name, 1902)  
(Fig. A)

**Tamanho:** Colônias incrustantes, delgadas (2,0 a 3,0mm de espessura), podendo alcançar 50cm de diâmetro. Zoóides com aproximadamente 1,0mm de comprimento.

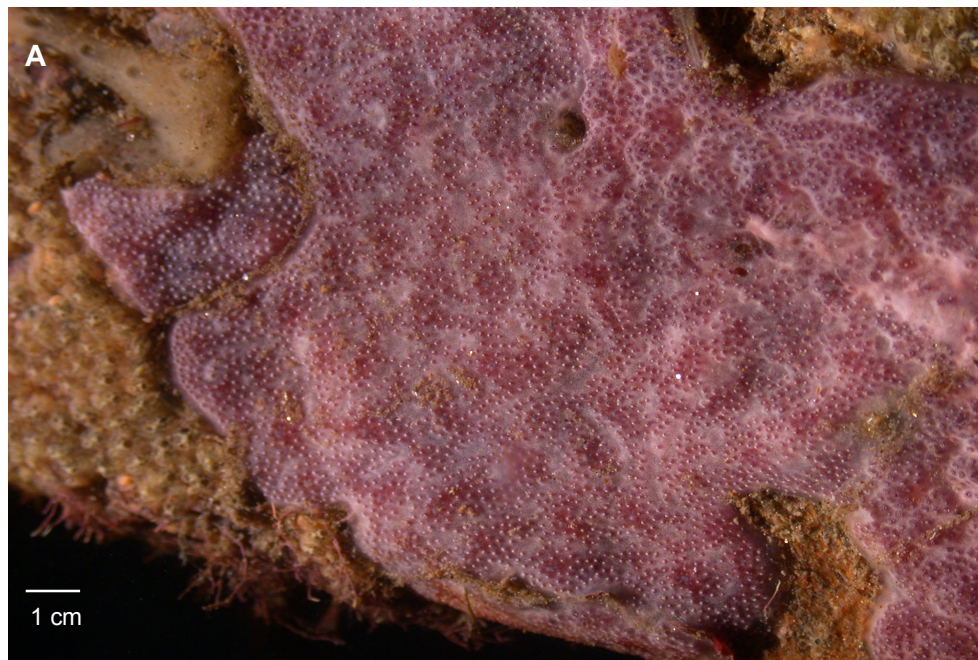
**Identificação:** Colônias de coloração arroxeadas, principalmente ao redor das cloacas, entremeada por pontuações brancas ou amareladas, resultantes do acúmulo de espículas. Estas diferem em abundância entre as colônias, resultando num gradiente de coloração, desde o ametista uniforme, (Fig. A) até aquelas com grandes manchas amareladas.

Zoóide com testículo apresentando 3 ou 4 lobos piriformes, envoltos por 4 ou 5 voltas do espermiduto. Ovários com 1 óvulo maduro e poucos pequenos. Larva com 3 papilas adesivas e 6 pares de ampolas.

**Comentários:** O único representante do gênero no litoral paulista pode ser facilmente identificado pelo seu aspecto externo e pelo número de lobos do testículo que em *Polysyncraton* é sempre igual ou maior que 3, enquanto que em *Didemnum* é igual a 1 ou 2.

**Habitat:** Região entremarés, principalmente próximo a franja do infralitoral, em fendas e sob rochas.

**Distribuição Geográfica:** Atlântico tropical americano.



*Clavelina oblonga* Herdman, 1880  
(Figs. A-B)

**Tamanho:** Colônias podendo atingir até 15cm de diâmetro. Zoóides grandes, medindo 2cm.

**Identificação:** Colônias formadas por até 50 zoóides, individualizados, presos pela base a um estolão, com ambos os sífões abrindo-se individualmente na superfície da colônia (Figs. A-B). Apresentam túnica transparente e gelatinosa, com sífões lisos e pigmentados de branco.

**Comentários:** Há na literatura registros de uma outra espécie pertencente ao gênero, *Clavelina picta*. Diferenças na coloração do zoóide vivo e no número destes na colônia separam, entretanto, as duas espécies. Segundo Lotufo (2002), provavelmente *Clavelina picta* não ocorre no litoral paulista.

**Habitat:** É comum no litoral paulista crescendo sobre rochas e pilares de atracadouros.

**Distribuição Geográfica:** Atlântico tropical e subtropical.





## *Distaplia bermudensis* Van Name, 1902

(Figs. A-D)

**Tamanho:** Colônias com até 6cm de diâmetro e 4 a 5mm de espessura. Zoóides com até 3mm de comprimento.

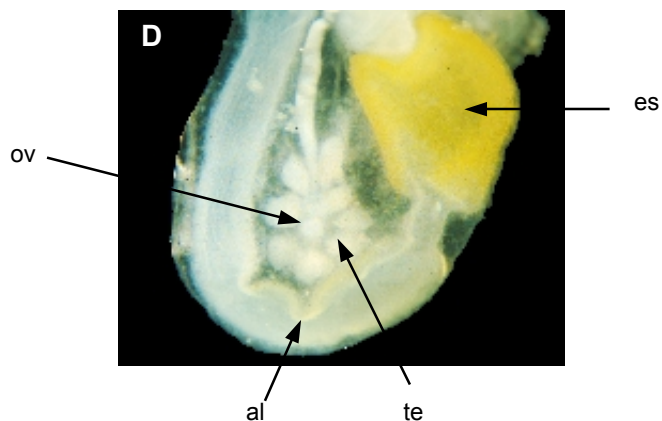
**Identificação:** Colônias de contorno arredondado, almofadadas, coloração muito diversificada, variando de brancas com manchas escuras próximo aos sifões (Fig. A) a esverdeadas (Fig. B), amareladas, alaranjadas, avermelhadas e rosadas (Fig. C).

Zoóide branco, facilmente removível da túnica. Abertura atrial ampla, expondo parte da cesta branquial, com bordo dorsal formando uma longa lingüeta. Trato digestório com estômago (es) amarelo e uma única alça (al) intestinal, junto à qual ocorrem gônadas em forma de roseta. Testículo (te) constituído por 6 a 8 lobos piriformes ao redor do ovário (ov) (Fig. D). Larva com 3 papilas adesivas dispostas de forma triangular, incubada em uma bolsa conectada ao zoóide.

**Comentários:** São animais de fácil manipulação, grandes, de coloração contrastante e de grande beleza, sendo ideais para o estudo da morfologia de zoóides em ascídias colônias.

**Habitat:** São encontradas pequenas colônias no entremarés crescendo sob rochas em pequenas áreas sombreadas. Na franja do infralitoral começam a se tornar maiores e mais abundantes.

**Distribuição Geográfica:** Atlântico tropical.



*Perophora multiclathrata* (Sluiter, 1904)

(Fig. A)

**Tamanho:** Colônias pequenas, nunca maiores que 3cm de extensão. Zoóides com 2,0 a 2,5mm de comprimento.

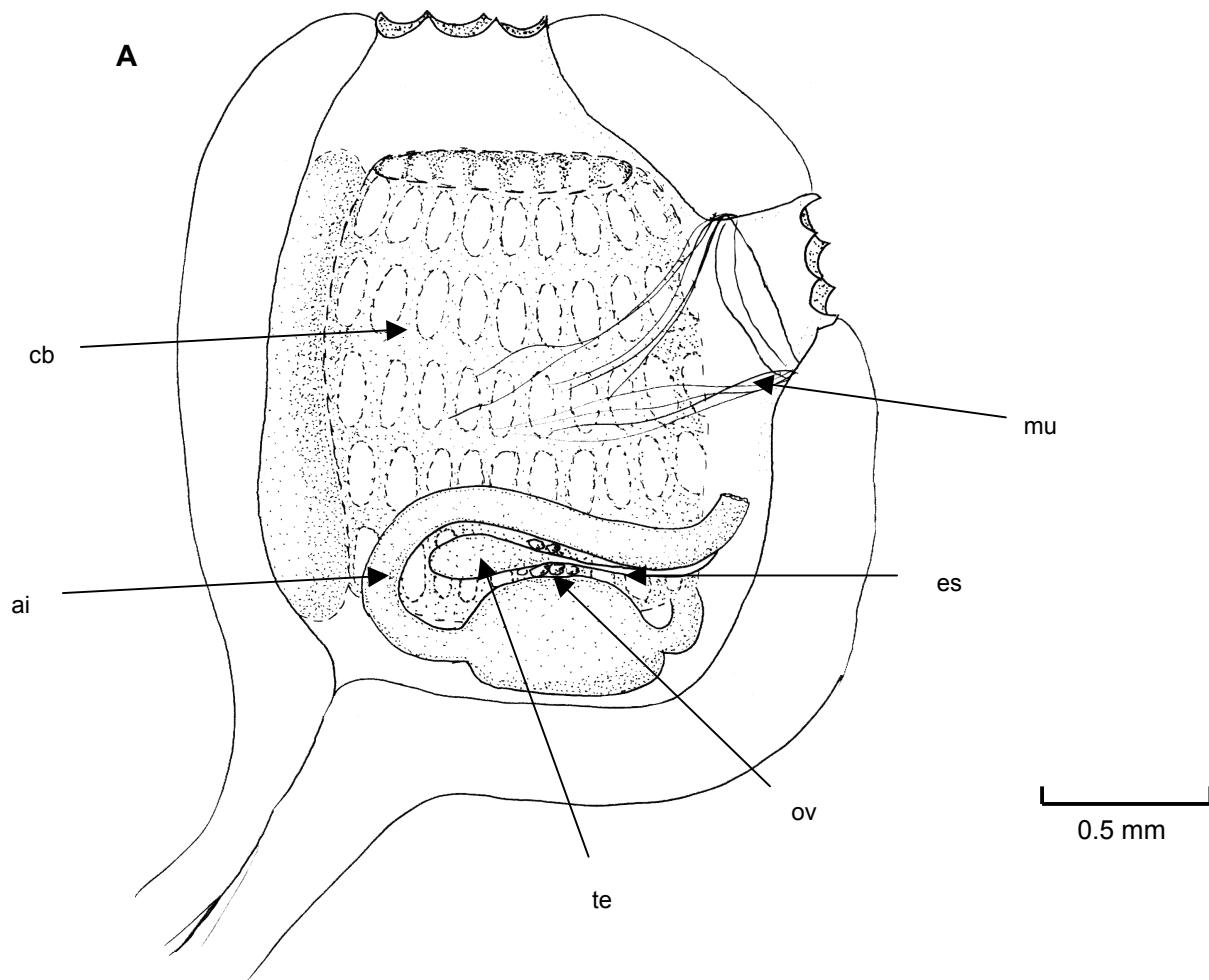
**Identificação:** Colônias formando estolões lineares ou ramificados. Túnica delicada e transparente, com tonalidade esverdeada.

Zoóide saculiforme ligado ao estolão por um pequeno talo. Apresenta musculatura (mu) formada por fibras que partem da região central de ambos os lados do corpo, passando pelo sifão atrial que as divide ao meio. Cesta branquial (cb) com 5 fileiras de fendas. Gônadas na alça intestinal (ai). Ovário (ov) situado em volta do espermiduto (es), na base do testículo (te) que é formado por um folículo piriforme (Fig. A).

**Comentários:** Esta espécie se diferencia de *Perophora viridis* por apresentar uma fileira de fendas branquiais a mais, pelo formato das gônadas e pela disposição da musculatura. Em animais fixados, não anestesiados, é comum a contração das fibras e em consequência disso há uma invaginação da túnica ao redor do sifão atrial, fazendo com que este fique pouco visível.

**Habitat:** Sob rochas na região entremarés, principalmente em locais abrigados com pouca movimentação de água.

**Distribuição Geográfica:** Pantropical



## *Perophora viridis* Verril, 1871

(Figs. A-C)

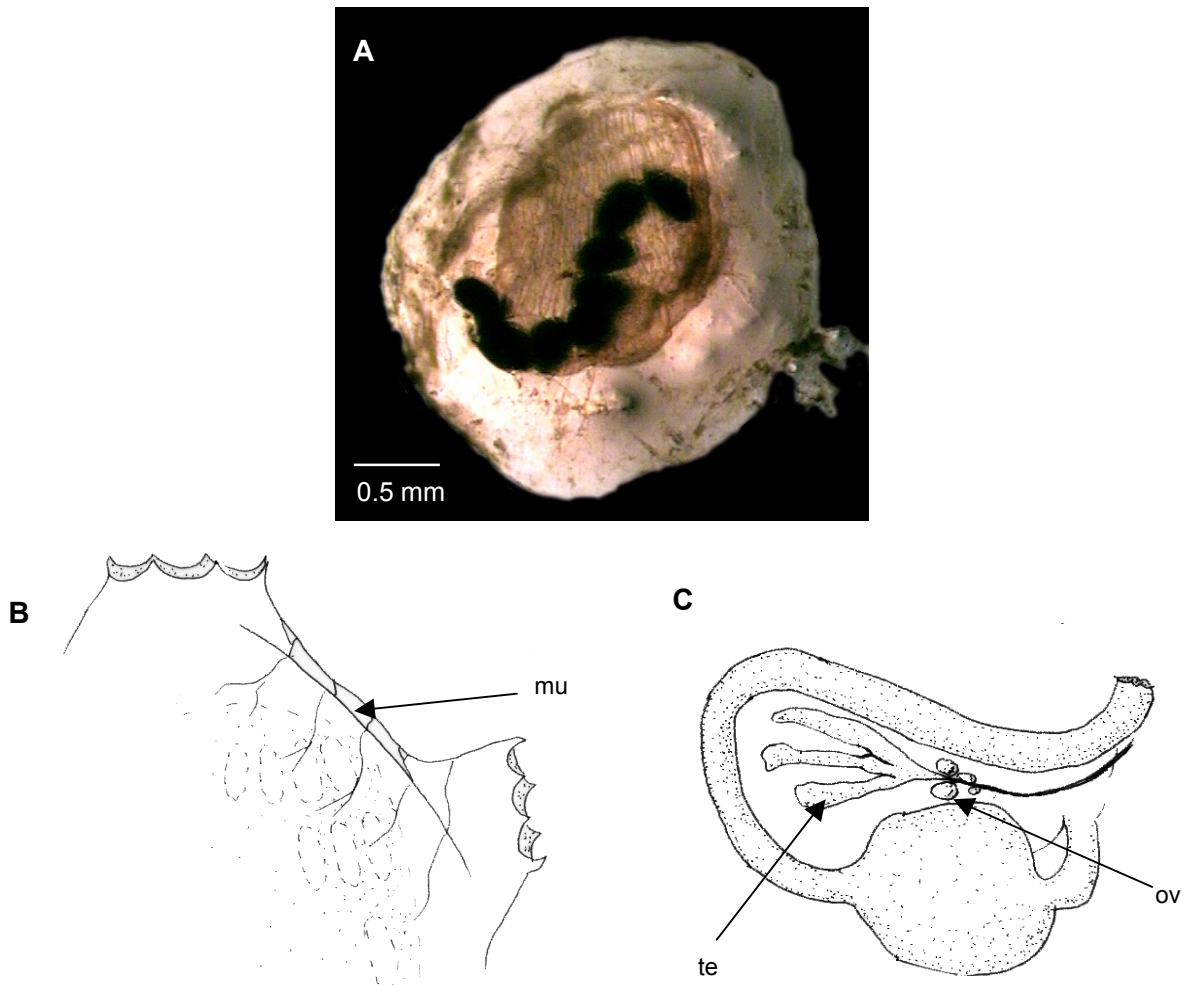
**Tamanho:** Colônias pequenas, raramente maiores que 15cm. Zoóides medindo até 2,5mm de comprimento.

**Identificação:** Colônias muito semelhantes às de *Perophora multiclathrata*, sendo necessário a observação dos zoóides (Fig. A) para determinação da espécie. Estes apresentam musculatura (mu) com dois feixes de fibras musculares, um de cada lado do corpo, localizados apenas entre os dois sífões (Fig. B). Cesta branquial com 4 fileiras de fendas. Ovário (ov) com 3 ou 4 óvulos ao redor do espermiduto na base do testículo (te), o qual apresenta 3 ou 4 lobos piriformes (Fig. C).

**Comentários:** Em zoóides fixados sem anestesia, a musculatura contrai, fazendo com que, além do sífão atrial, a região entre os dois sífões também invagine, formando um sulco que se prolonga até o sífão branquial, o qual, algumas vezes, também é observado retraído.

**Habitat:** Sob rochas na região entremarés, principalmente em locais abrigados.

**Distribuição Geográfica:** Oceano Atlântico tropical e subtropical.



## *Botryllus tabori* Rodrigues, 1962

(Figs. A - D)

**Tamanho:** Colônias delgadas, com 0,2 a 0,4 mm de espessura. Sistemas alcançando 3,5cm de comprimento. Zoóides com 0,8 a 1,5mm de comprimento.

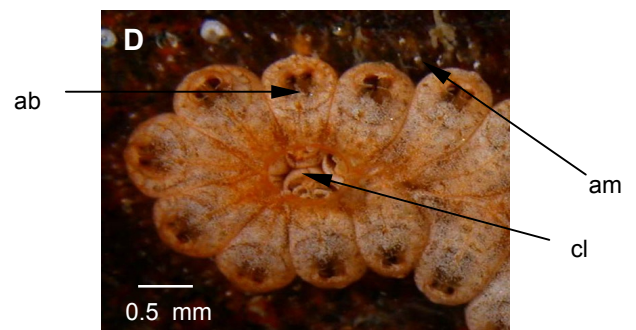
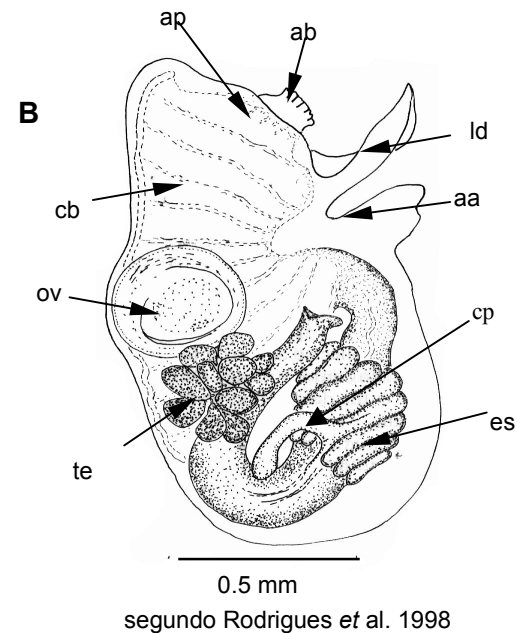
**Identificação:** Colônias incrustantes e achatadas de coloração homogênea, variando do vermelho ao amarelo, às vezes arroxeadas ou marrons. Zoóides dispostos em torno de uma cavidade cloacal (cl) (Fig. D) formando sistemas lineares (Fig. C) ou ramificados, os quais podem ser alongados ou elípticos (Fig. A). Zoóide facilmente observado a olho nu, embebido em túnica comum, com limites bem definidos e sífões branquiais e cloacas destacando-se pouco da superfície da colônia (Fig. D). Vasos sanguíneos ramificados, terminando em ampolas conspícuas (am) (Fig. D), geralmente na borda da colônia.

Para confirmação da espécie é recomendado a dissecação do zoóide (Fig. A), o qual apresenta abertura branquial (ab) circular, abertura atrial (aa) com uma pequena lingüeta dorsal (ld), anel perifaríngeo (ap) com 8 a 12 tentáculos orais, cesta branquial (cb) com 6 a 9 fileiras de fendas, estômago (es) com 9 dobras longitudinais e ceco pilórico evidente (cp), testículo (te) com 5 a 12 lobos e ovário (ov) com 1 ou 2 óvulos (Fig. 1B).

**Comentários:** Esta espécie pode ser confundida com *Botryllus humilis*, cuja falta de material dificulta comparações. *Botryllus humilis*, entretanto, apresenta duas manchas brancas no bordo dos sífões, o que lhe proporciona um aspecto não uniforme.

**Habitat:** Região entremarés de costões rochosos, abundantes sob rochas, sobre algas, esponjas e ascídias solitárias.

**Distribuição Geográfica:** Litoral brasileiro.



## *Botrylloides giganteum* (Pérès, 1949)

(Figs. A-B)

**Tamanho:** Colônias carnosas, com até 1cm de espessura (Fig. B), podendo atingir 40cm de comprimento máximo. Zoóides grandes, medindo de 2,5 a 4,0cm de comprimento.

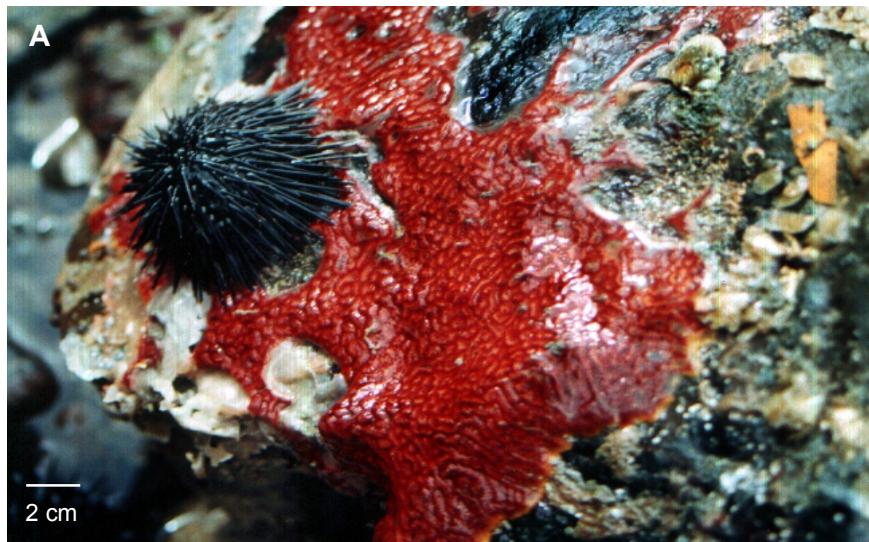
**Identificação:** Colônias de coloração alaranjada, vermelha ou arroxeadas, formada por zoóides justapostos, nunca formando sistemas lineares distintos como em *B. tabori* (Fig. A). Túnica resistente e de coloração não homogênea.

Zoóide apresentando de 14 a 17 fileiras de fendas na cesta branquial, facilmente observadas sob estereomicroscópio.

**Comentários:** Esta espécie, até 1991, era considerada como uma variedade de *Botrylloides nigrum*. Aron & Solé-Cava (1991) utilizando-se de caracteres morfológicos e análise isoenzimática a elevaram à condição de espécie.

**Habitat:** Desde a região entremarés até pequenas profundidades.

**Distribuição Geográfica:** Costa oeste e sudeste africana, Golfo do México e sudeste brasileiro.



***Botrylloides nigrum* Herdman, 1886**

(Fig. A)

**Tamanho:** Colônias delgadas de 0,2 a 0,3 mm de espessura, alcançando 15cm de comprimento. Zoóides com 1,5 a 2,0 mm de comprimento.

**Identificação:** Colônias incrustantes e achatadas de coloração marrom-arroxeadada com uma mancha amarelo alaranjada, peculiar, em forma de ferradura ao redor do sifão branquial (Fig. A). Colônias apresentando formas variadas, acompanhando o substrato, formando sistemas de zoóides alongados ou elípticos, em geral compactos.

**Comentários:** Esta é a mais conspícua dentre as espécies coloniais, sendo comumente encontrada no litoral paulista.

**Habitat:** Região entremarés de costões rochosos, abundante em fendas ou sob rochas, sendo comumente encontrada sobre algas e outros organismos.

**Distribuição Geográfica:** Pantropical, comum em todo o litoral tropical brasileiro.



## *Botryllus tuberatus* Ritter & Forsyth, 1917

(Figs. A-C)

**Tamanho:** Colônias podendo alcançar 40cm de diâmetro. Zoóides com até 1mm de comprimento.

**Identificação:** Colônias com túnica transparente, o que permite a visualização dos zoóides dourados ou marrons. Estes estão organizados em sistemas, como em *Botryllus tabori*, porém em menor número (8 a 13) (Fig. C).

Zoóide apresentando cesta branquial com 4 fileiras de fendas. Estômago em forma de barril com 8 dobras longitudinais.

**Comentários:** Esta espécie é muito delicada e de difícil remoção da rocha, porém é inconfundível devido a sua coloração, podendo ser reconhecida ainda a olho nu.

**Habitat:** Encontrada sob rochas e em talos de algas (Figs. A-B), principalmente na franja do infralitoral.

**Distribuição Geográfica:** Pantropical



*Eusynstyela tincta* (Van Name, 1902)  
(Figs. A-B)

**Tamanho:** Colônias com até 5cm de diâmetro. Zoóides com até 1cm de comprimento.

**Identificação:** Colônias formadas por poucos zoóides achatados dorso-ventralmente, alongados, de coloração avermelhada, eventualmente recobertos por sedimento (Figs. A-B). Zoóide de manto frágil, amarelado e com muitos endocarpos e cesta branquial com 4 pregas de cada lado. Estômago alongado com dobras longitudinais. Gônadas arredondadas constituídas por duas fileiras de oito, uma de cada lado do endóstilo.

**Comentários:** As pequenas colônias desta espécie quando removidas da água são reconhecidas pelos sífões avermelhados e pela delimitação nítida dos zoóides.

**Habitat:** Sob rochas na região entremarés.

**Distribuição Geográfica:** Encontrada desde a Flórida (EUA) até o Estado de São Paulo (Brasil) e no litoral tropical africano.





## *Polyandrocarpa zorritensis* (Van name, 1931)

(Figs. A-B)

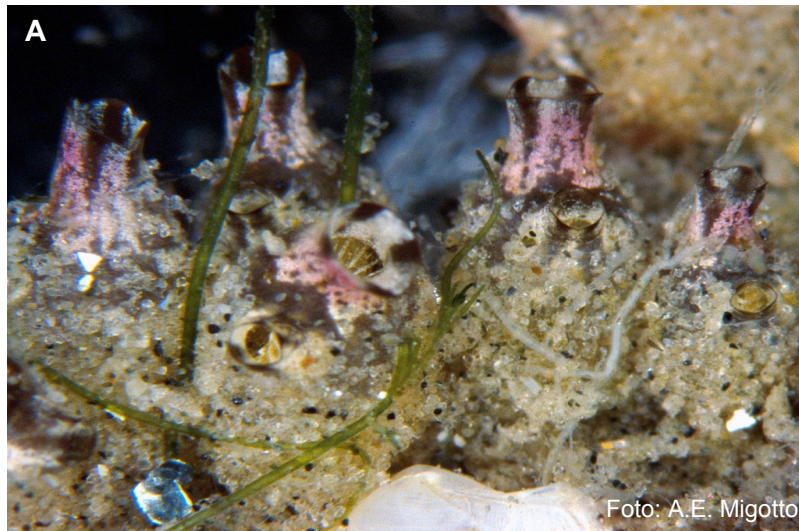
**Tamanho:** Colônias ultrapassando 10cm de diâmetro formadas por zoóides de até 1cm de comprimento.

**Identificação:** Zoóides delicados, bem individualizados, unidos pela base, de coloração castanha ou marrom. Sifões quadrilobados de pigmentação mais escura, protuberantes na superfície da colônia. Túnica sempre incrustada por areia, facilmente removível, expondo um manto translúcido levemente amarelado. Três a oito gônadas, características da espécie, arredondadas com ambos os sexos, em cada lado do corpo, paralelas ao endóstilo (Figs. A-B).

**Comentários:** A observação da disposição das gônadas bem como o aspecto externo da colônia são fundamentais para a determinação da espécie.

**Habitat:** É encontrada desde o entremarés até pequenas profundidades crescendo sob e sobre rochas ou substratos artificiais.

**Distribuição Geográfica:** Brasil, Peru e Japão.



## *Symplegma brakenhielmi* (Michaelsen, 1904)

(Figs. A-B)

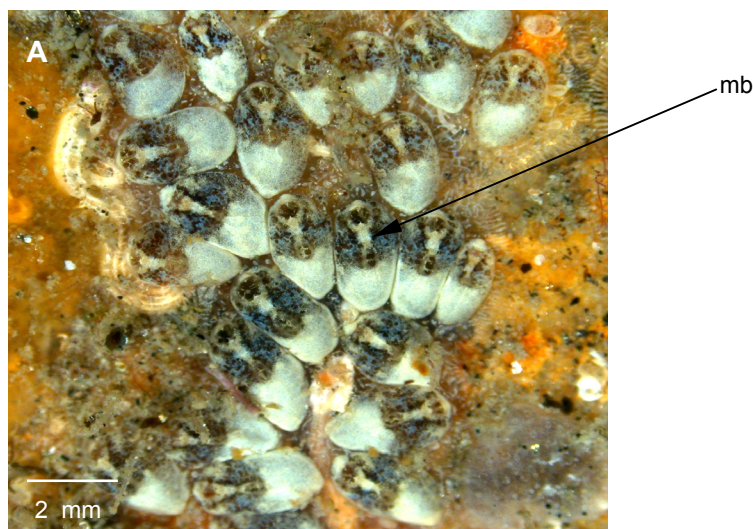
**Tamanho:** Colônias incrustantes com 2 mm de espessura, em geral medindo de 8 a 10cm de comprimento, podendo, no entanto, alcançar 20cm ou mais. Zoóides medindo de 2 a 4 mm.

**Identificação:** Colônias de coloração amarela, esverdeada ou levemente azulada (Figs. A-B), apresentando manchas brancas, vermelhas ou pretas entre os sífões (mb), nunca com o anel vermelho unindo os dois sífões, como em *Symplegma rubra*. Túnica delicada e transparente, zoóide achatado dorso-ventralmente. Uma gônada de cada lado do corpo, com 2 folículos lobados piriformes e um ovário com dois óvulos.

**Comentários:** Durante a revisão do gênero *Symplegma*, Couto (2003) propôs a separação dos dois morfotipos, representados nas figuras A e B, em duas espécies diferentes, sendo o primeiro, de coloração azulada com uma mancha branca entre os sífões, mantido como *Symplegma brakenhielmi*. O morfotipo verde amarelado, com uma mancha vermelha entre os sífões foi descrito como uma espécie nova.

**Habitat:** Ocupa todo o entremarés, raramente ultrapassando a franja do infralitoral, onde é muito freqüentes sob pedras soltas e fendas de rochas.

**Distribuição Geográfica:** Atlântico tropical americano



## *Symplegma rubra* Monniot C., 1972

(Figs. A-D)

**Tamanho:** Colônias incrustantes com 2mm de espessura, de tamanho variado, atingindo até 70cm de comprimento. Zoóides medindo de 2 a 4mm de comprimento.

**Identificação:** Colônias incrustantes e achatadas, de coloração viva, uniforme e muito variável, podendo ser amarela, laranja, cor de tijolo, carmim, rosa e vermelha (Figs. A-B-D). Geralmente apresentam um anel vermelho (av) que passa pelos dois sífões (Fig. C). Túnica transparente e delicada. Zoóide achatados dorso-ventralmente, de forma oval, com os dois sífões iguais se abrindo na superfície da colônia (Figs. A-B-D).

**Comentários:** É comum em zoóides de coloração vermelha a não visualização do anel acima descrito. Neste caso, a principal diferença entre esta espécie e *S. brakenhielmi* é o formato dos testículos, palmados na primeira e piriformes na última.

**Habitat:** Ocorre desde a zona entremarés, até alguns metros de profundidade, sendo comumente encontrada em faces inferiores e laterais de rochas.

**Distribuição Geográfica:** Atlântico tropical americano.

