

Introduced Ascidians in Paranaguá Bay, Paraná, southern Brazil ¹

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ABSTRACT. Exotic (introduced) species are a growing problem in ports worldwide and comprise the most important impacts in marine ecosystems. Periodic monitoring to detect introduced species is extremely important for effective population control. Here we sampled ascidian species near the port of Paranaguá for a taxonomic study of this fauna to attempt to detect introduced species. Larval stages in ascidians are short-lived, and dispersal is restricted to small distances, and so ascidians are very good bioindicators for exotic introductions due to ship transport. Four locations were sampled within Paranaguá Bay (Ilha das Cobras, Pier Tenenge, Ilha do Mel and Ilha da Galheta) and one location outside of the bay (Parque dos Meros). Information for the nearby fauna and for geographic distributions of the species involved was obtained from the literature. Eighteen species were found: *Perophora multiclathrata* (Sluiter, 1904), *Ascidia curvata* (Traustedt, 1882), *A. sydneiense* Stimpson, 1855, *Clavelina oblonga* Herdman, 1880, *Cystodytes dellechiaiei* (Della Valle, 1877), *Eudistoma carolinense* van Name, 1945, *Distaplia bermudensis* van Name, 1902, *Didemnum granulatum* Tokioka, 1954, *Diplosoma listerianum* (Milne-Edwards, 1841), *Lissoclinum fragile* (van Name, 1902), *Botryllus planus* (van Name, 1902), *B. tuberatus* Ritter & Forsyth 1917, *Botrylloides nigrum* Herdman, 1886, *Symplegma rubra* Monniot, 1972, *Styela canopus* (Savigny, 1816), *S. plicata* (Lesueur, 1823), *Microcosmus exasperatus* Heller, 1878 and *Molgula phytophila* Monniot, 1970. The known geographic distributions based on the literature and collections suggest that three species are native, one is a inter-regional introduction, two are introduced from the Pacific and the remaining 12 are cryptogenic.

KEY WORDS. Ascidiacea, Tunicata, introduced species, bioinvasion.

RESUMO. **Ascídias introduzidas na Baía de Paranaguá, Paraná, sul do Brasil.** A crescente presença de espécies introduzidas (exóticas) em portos é um dos maiores problemas ambientais que vêm sendo enfrentados nos ecossistemas marinhos. O monitoramento periódico da fauna é necessário para uma detecção precoce das espécies introduzidas. O objetivo deste trabalho é verificar a presença de espécies não nativas na região de influência do Porto de Paranaguá, por meio de estudo taxonômico da fauna de ascídias desta região. As ascídias possuem larvas de curta duração, fazendo com que sua dispersão natural fique restrita a pequenas distâncias. Assim, os representantes desta Classe podem constituir ótimos bioindicadores para introduções mediadas pelo transporte de grandes navios. Foram realizadas coletas em quatro localidades na Baía de Paranaguá (Ilha das Cobras, Pier Tenenge, Ilha do Mel e Ilha da Galheta) e uma fora da Baía (Parque dos Meros) e as informações sobre a fauna de Ascidiacea de outras ilhas próximas, bem como a distribuição geográfica das espécies, foram obtidos na literatura. Identificou-se 18 espécies de ascídias: *Perophora multiclathrata* (Sluiter, 1904), *Ascidia curvata* (Traustedt, 1882), *A. sydneiense* Stimpson, 1855, *Clavelina oblonga* Herdman, 1880, *Cystodytes dellechiaiei* (Della Valle, 1877), *Eudistoma carolinense* van Name, 1945, *Distaplia bermudensis* van Name, 1902, *Didemnum granulatum* Tokioka, 1954, *Diplosoma listerianum* (Milne-Edwards, 1841), *Lissoclinum fragile* (van Name, 1902), *Botryllus planus* (van Name, 1902), *B. tuberatus* Ritter & Forsyth 1917, *Botrylloides nigrum* Herdman, 1886, *Symplegma rubra* Monniot, 1972, *Styela canopus* (Savigny, 1816), *S. plicata* (Lesueur, 1823), *Microcosmus exasperatus* Heller, 1878 and *Molgula phytophila* Monniot, 1970. Levando em consideração a distribuição geográfica da espécie, o registro histórico da região e informações provenientes da literatura conclui-se que três espécies podem ser classificadas como nativas, uma como uma introdução inter-regional, duas como introduzidas e 12 como criptogênicas.

PALAVRAS CHAVE. Ascidiacea, Tunicata, espécies introduzidas, bioinvasão.

Increasingly, invasive marine species are being reported in ports throughout the world. Such introductions may, and often do, create large environmental problems, such as competition for limited resources, exclusion of native species, the transmission of parasites and diseases, and economic problems, such as those associated with shellfish cultivation. In Tasmania, the introduced predatory seastar (*Asterias amurensis*) is responsible for the reduction and rarity in bivalve species that live just below or on the sediments in the Derwent Estuary. Impacts on native assemblages, wild fisheries and mariculture operations in areas outside the Derwent Estuary are of immediate management concern (ROSS *et al.* 2003). Another example, the polychaete *Terebrasabella heterouncinata* arrived as an epizoic on South African abalone imported to California for commercial aquaculture research in 1993. Its unique mechanism of settlement on the outer lip of the shell aperture altered calcium deposition and often severely retarded the growth of infested individuals and economic losses to the industry were considerable (CULVER & KURIS 2000). The kelp *Undaria pinnatifida* (Phaeophyceae), native to northeast Asia, was first detected in late 1992 offshore in central Patagonia (Argentina) and is progressively spreading, along with a dramatic decrease in species richness and diversity of native seaweeds in Nuevo Gulf (CASAS *et al.* 2004). These are only a few recent examples.

Most introductions are new invading species carried by ocean-going vessels, encrusted on their hulls, in their ballast waters or seachests. Therefore, ports are often the principal areas of arrival of new invasive species (CARLTON & GELLER 1993, RUIZ *et al.* 2000, WASSON *et al.* 2001), though a number of species have been introduced with imported shellfish as epibiotic foulers, an often overlooked transport vector (LAMBERT 2005b). Once arrived, new species may find the areas in and around ports to be favorable for their growth and establishment (calm waters with many man-made structures suitable for colonizing and often nutrient rich due to pollution). In these conditions, invasive species populations often grow quickly.

Ascidians are among the many organisms that are passively transported in this way. As sessile benthic filter-feeders, these species may often find suitable habitat, at least if the port is not estuarine. Also, ascidians are hermaphroditic and often able to self-fertilize, they have rapid growth rates and a long fertile period, and therefore may reproduce at very high rates (BERRILL 1975, see review by LAMBERT 2005b). Also, being able to regenerate from fragments (BERRILL & COHEN 1936, TURON 1992) is another characteristic that favors their establishment in new locations. Many ascidians are viviparous (all colonial and some solitary species) and larvae are protected in incubation sacs or in the tunic during their development, after which they are released to the environment. These larvae are lecithotrophic and have short, free-swimming periods, during which they must find a substrate on which to anchor themselves (MILLAR 1971, MONNIOT *et al.* 1991). Due to these characteristics, dispersal is limited, while population growth may be rapid. Thus, ascidians have naturally

limited distributions, and the widespread appearance of some species may indicate that they have been introduced by man.

Several ascidian species are known as introductions. *Perophora japonica*, from the northwest Pacific Ocean is now found in Europe (MONNIOT & MONNIOT 1985, BALDOCK & BISHOP 2001) and in 2003 was found in northern California – a first record for North America (LAMBERT 2005a). *Styela clava*, also from the northwest Pacific Ocean, is also found in Europe (DAVIS & DAVIS 2005 for a recent review) and the United States (LAMBERT & LAMBERT 1998, 2003). *Molgula manhattensis* went the other way, originating in the North American Atlantic coast, was found in Japan and Australia in the 1970s, and in Vladivostok in 1999 (ZVYAGINTSEV *et al.* 2003). *Ciona intestinalis*, widespread in the Northern Hemisphere, has been introduced into many of the larger ports of the Southern Hemisphere, including both east and west coasts of Australia (KOTT 1990, McDONALD 2004). In 2002, the southern hemisphere species *Corella eumyota* was found in the northern hemisphere for the first time, attached to floating docks in two harbors in northwestern France (LAMBERT 2004). *Polyandrocarpa zorritensis*, originally described in Peru, is now found in the Mediterranean Sea (BRUNETTI 1978-79, BRUNETTI & MASTROTOTARO 2004), in Japan (NISHIKAWA *et al.* 1993) and in California (LAMBERT & LAMBERT 2003). Atlantic populations of *Clavelina lepadiformis* have been found in the Mediterranean Sea, transported on the hulls of ships (TURON *et al.* 2003). From this small compilation, it is clear that ascidians have been carried distances both small and large, between hemispheres and throughout the world. Species of any Order, solitary and colonial, are all subject to transportation and introduction.

In the state of Paraná, the port of Paranaguá (25°30,1' S; 48°31,0' W), the most important port in southern Brazil, receives ships that transport grain (mostly soy), chemicals, oil and industrial goods from and to many parts of the world. The number of ships passing through this port each year is growing annually: from 1,737 ships in 2000 to 2,204 in the year 2004 (<http://www.pr.gov.br/portos>, in 11/24/2005). In March 2001 a toxic algae bloom occurred near Laranjeiras Bay (within Paranaguá Bay), and was the first ever reported, causing serious economic impact on the local fishing industry (L. F. FERNANDES 2001, personal communication). *Coscinodiscus walesii* is another new algal species for South American, and recently found in Paranaguá Bay (FERNANDES *et al.* 2001). A species of ascidian (*Bostricobranthus digonas*, Abbott 1951) was described from Florida, in the United States, and was also found in Paranaguá Bay probably introduced, since it has not yet been found again on subsequent attempts (ROCHA 2002). Thus, Paranaguá Bay is now host to a variety of introduced species. In Brazil, eight species of ascidians were potentially introduced in the historical past: *Ciona intestinalis*, *Phallusia nigra*, *Didemnum perlucidum*, *Diplosoma listerianum*, *Microcosmus exasperatus*, *Styela canopus*, *Styela plicata* and *Symploca brakenhielmi*.

With these considerations, our goal in this study was to sample the ascidian community in the area of influence of the

Port of Paranaguá. Specifically, we wished to use ascidians as indicator species to better understand the biology of species introductions due to shipping in marine systems.

MATERIAL AND METHODS

Five locations were sampled within and near Paranaguá Bay (Fig. 1): Ilha das Cobras (25°29,00'S; 48°25,50'W) and Pier Tenenge (25°32,84'S; 48°21,51'W), sampled on 7 December 2003; Ilha da Galheta (25°35,00'S; 48°19,00'W) on 11 March 2004; Ilha do Mel (25°33,00'S; 48°18,00'W) on 11 March 2004 and 9 June 2005; and Mero Park (25° 43,00' S; 48° 20,00' W) on 14 May 2005. Ascidians were collected by scuba diving at depths of up to four meters, except Mero Park, where depths reached a maximum of 17 m. Samples were anesthetized and fixed in 10% neutralized formalin.

Since historical records of ascidian species are scarce, we decided whether species were native, introduced or cryptogenic (of unknown status) based on their published geographic records, by exhaustively searching the literature for all publications that included distributional (latitude and longitude) information, along with habitat and substrate type, for all of the ascidian species encountered. Cryptogenic species were considered to be those species with widespread distributions and without information of known native distributions in the literature, as well as Atlantic species with disjunct distributions between northern and southern hemispheres. We considered introduced species as those with worldwide distributions but are either unknown from northeastern Brazil, or have strong indications of introduction in other locations in the literature, or only occur on artificial substrates in southern Brazil.

RESULTS

Eighteen species (Tab. I) were collected, with 30 samples from Ilha das Cobras, 26 from Pier Tenenge, nine from Ilha do Mel, 11 from Ilha da Galheta and five from Parque dos Meros. Only six samples were of solitary animals, collected at Ilha da Galheta, Ilha do Mel and Mero Park. Some samples in the family Didemnidae are still unidentified, since larvae (which have characters of critical importance in species identification) were absent.

Four distinct distribution patterns were found for this ascidian community. First are the endemics and included only one species, *Molgula phytophila* (Fig. 2). Next, species found from the Caribbean to southern Brazil (*Ascidia curvata*, *Eudistoma carolinense*, *Botryllus planus*; Figs 3-5). Third, species found in the Caribbean and in southern Brazil, but not recorded in northeastern Brazil (*Ascidia sydneyensis*, *Styela plicata*; Figs 6-10). Fourth, very widespread species, which we call cryptogenic (*Clavelina oblonga*, *Distaplia bermudensis*, *Cystodytes dellechiaiei*, *Didemnum granulatum*, *Lissoclinum fragile*, *Diplosoma listerianum*, *Perophora multiclathrata*, *Botrylloides nigrum*, *Botryllus tuberatus*, *Symplegma rubra*, *Styela canopus*, *Microcosmus exasperatus*; Figs 11-19).

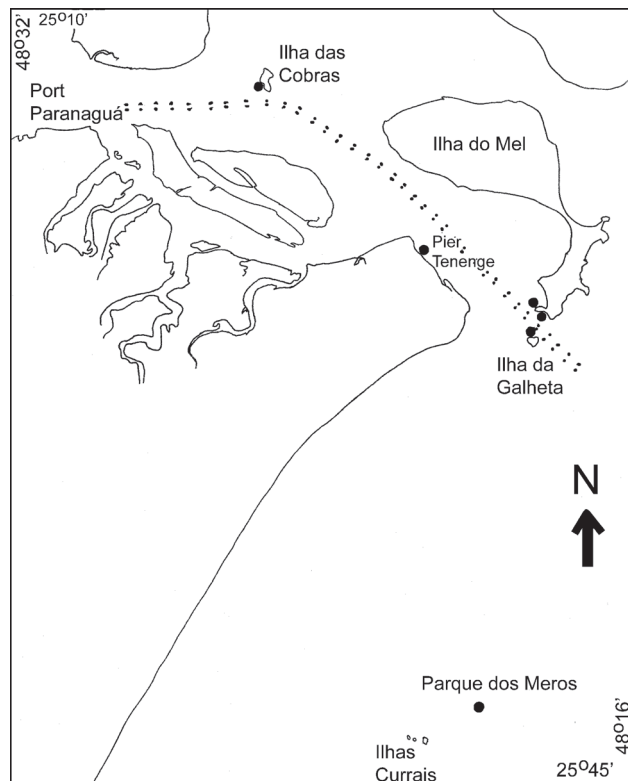


Figure 1. Map of the region of Paranaguá Bay, and collecting locations (marked with black circles). The dotted line indicates the canal through which cargo ships pass on their way to port.

DISCUSSION

Molgula phytophila, the only endemic species in the region (Fig. 2, Tab. II), was found encrusted on fish-nets in the concrete blocks that form the artificial reef in Mero Park. It is quite possible that this species is synonymous with *M. braziliensis* Millar, 1958, but the type specimen (London Natural History Museum) of the latter is of poor quality and cannot be used for identification of the former. If synonymous, the specimen in MILLAR (1958) would be the first recorded in the Brazilian coast.

Ascidia curvata, *Eudistoma carolinense* and *Botryllus planus* have a continuous distribution from the Caribbean to our study area, and also present some records in North America (Figs 3-5, Tab. II). This distribution suggests that these species are native, with a distribution that includes the western Atlantic Ocean. However, the status of the species with this distribution has been debated. The Caribbean fauna should extend only to the Brazilian state of Espírito Santo, following PALÁCIO (1982), who considered the region between Espírito Santo and the southernmost Brazilian state of Rio Grande do Sul, as a region of high endemism and therefore a biogeographical province.

Table I. List of the species and occurrences at each sampling site.

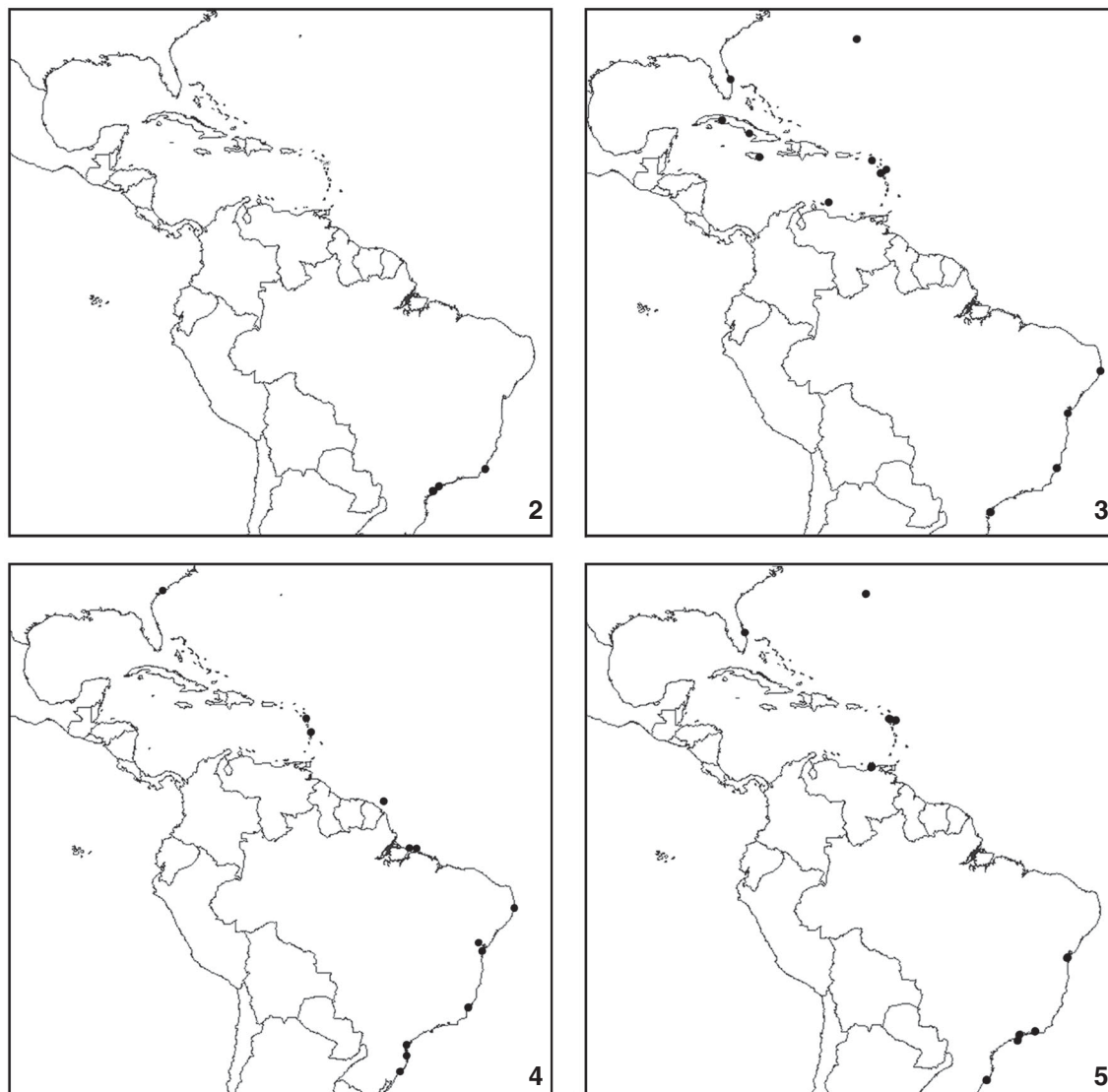
	Ilha das Cobras	Pier Tenenge	Ilha do Mel	Ilha Galheta	Parque dos Meros
Perophoridae					
<i>Perophora multiclathrata</i> (Sluiter, 1904)	X				
Asciidiidae					
<i>Ascidia curvata</i> (Traustedt, 1882)				X	
<i>Ascidia sydneiensis</i> Stimpson, 1855				X	
Clavelinidae					
<i>Clavelina oblonga</i> Herdman, 1880	X	X	X	X	
Polycitoridae					
<i>Cystodytes dellechiaiei</i> (Della Valle, 1877)				X	
<i>Eudistoma carolinense</i> van Name, 1945			X	X	
Holozoidae					
<i>Distaplia bermudensis</i> van Name, 1902	X	X			
Didemnidae					
<i>Didemnum granulatum</i> Tokioka, 1954		X	X		
<i>Diplosoma listerianum</i> (Milne-Edwards, 1841)				X	
<i>Lissoclinum fragile</i> (van Name, 1902)		X			
Styelidae					
<i>Botryllus planus</i> (van Name, 1902)	X	X			
<i>Botryllus tuberatus</i> Ritter e Forsyth, 1917				X	X
<i>Botrylloides nigrum</i> Herdman 1886			X		
<i>Symplegma rubra</i> Monniot C., 1972	X	X		X	
<i>Styela canopus</i> (Savigny, 1816)					X
<i>Styela plicata</i> (Lesueur, 1823).			X		
Pyuridae					
<i>Microcosmus exasperatus</i> Heller, 1878					X
Molgulidae					
<i>Molgula phytophila</i> Monniot, 1969-70			X		X

However, a study of the gastropod community did not support this hypothesis, since endemism was not high within these latitudes, which should therefore only be considered a region of transition (FLOETER & SOARES-GOMES 1999). Also, the Brazilian Current reaches the state of Santa Catarina, providing a dispersal route and explaining southern ascidian distributions. In the north, the Gulf Current of the Caribbean works in the same way, carrying ascidians along and thereby explaining northern distribution patterns and the similarities between Bermuda and Caribbean ascidian faunas (MONNIOT & MONNIOT 1983).

Ascidia curvata was first reported as adults in Brazil in Paraná (ROCHA & NASSER 1998) while juveniles were collected in São Paulo in the 1990s that may be this species (RMR, unpublished data). *Eudistoma carolinense* was first reported in Brazil on the coast of the state of Pará (MILLAR 1977). This species has never been reported on artificial substrates, and thus may be classified as native, at least in northeastern Brazil. On the other hand, the lack of records of this species in the states of Rio de

Janeiro and São Paulo, suggests that the southern distribution may be explained by human transport. *Botryllus planus* was first reported in Brazil in Rio de Janeiro and São Paulo (COSTA 1969).

Ascidia sydneiensis and *Styela plicata*, all from other regions in the world, have disjunct distributions (type three) and so were considered introduced in Paraná (Figs 6-7, Tab. II). In Brazil, they have only been reported from the south and southeast with one report of *S. plicata* from Bahia (T. LOTUFO 2002, personal communication). *Ascidia sydneiensis* (first reported in São Paulo, BJORNBERG 1956) is rare in southeastern and southern Brazil and is almost never found on natural substrates (the exception being this study – the only individual was found underside a small boulder at Ilha da Galheta). The few other reports are on artificial substrates, such as ship hulls (MILLAR 1958) and in shellfish cultivation (Rocha, unpublished observations on shells of *Nodidipeten nodosus* (Linnaeus, 1758) at Penha, Santa Catarina). It was also introduced in Guam (LAMBERT 2003), Papeete (MONNIOT *et al.* 1985), Sierra Leon (MILLAR 1956, MONNIOT & MONNIOT 1994).



Figures 2-5. Distributions of the species found in Paranaguá Bay and nearby, with exclusive Atlantic distributions: (2) *Molgula phytophila*; (3) *Ascidia curvata*; (4) *Eudistoma carolinense*; (5) *Botryllus planus*.

Styela plicata is included with the “ascidan fauna inhabiting harbours and other largely man-made habitats throughout the tropical oceans” (MONNIOT & MONNIOT 1997). This species has been found in Brazil since the 19th century, with a record by TRAUSTEDT (1883) in Rio de Janeiro. The reason to include it as an introduced species is its rarity on natural substrates and its absence in northeastern Brazil (except for one individual in Marina de Aratu, Bahia; T. LOTUFO 2002, personal communication). Also, it is very common on artificial substrates in Rio de Janeiro, where it is one of the commonest fouling organisms on buoys and other floating structures (A. JUNQUEIRA 2005, personal communication). It is also very common on mussels in cultivation and on nets used for oyster cultivation

in the state of Santa Catarina, where it may occur in such large quantities that may cause competition with mussels and oysters as well as creating the need for costly cleaning of the cultivation structures. In São Sebastião, São Paulo, this ascidian was found on fishing nets (RODRIGUES 1962), and only once on natural substrates (ROCHA, non published observation). Interestingly, it was first described based on a sample taken from a ship hull in Philadelphia, Pennsylvania, in the United States, while not being found on natural substrates in the same region (VAN NAME 1945). Proof that this species travels on ship hulls is plentiful: “...from the bottom of the USS Palos after a Voyage across the Pacific from China or Japan” (TORIKOKA 1967).

Twelve species are cryptogenic: *Clavelina oblonga*,

Table II. List of ascidian species, and where found, on the Brazilian coast.

Species	Coastal Brazilian State										References	
	SC	PR	SP	RJ	ES	BA	AL	PE	RN	CE		PA
<i>Perophora multiclathrata</i>			X		X		X					11, 13
<i>Ascidia curvata</i>		X			X	X		X				10, 13
<i>Ascidia sydneiensis</i>	X	X	X	X								2, 3, 4, 10, 11, 14
<i>Clavelina oblonga</i>	X	X	X	X	X							2, 3, 4, 8, 10, 11, 13, 14, 15
<i>Cystodytes dellechiaiei</i>	X	X		X		X	X	X	X	X	X	1, 7, 10, 13, 14, 15
<i>Eudistoma carolinense</i>	X				X	X		X		X	X	7, 12, 13, 15
<i>Distaplia bermudensis</i>	X	X	X	X	X						X	3, 7, 8, 10, 11, 13, 14, 15
<i>Didemnum granulatum</i>	X	X	X			X			X	X		9, 11, 13, 15
<i>Diplosoma listerianum</i>	X	X	X	X	X	X	X	X	X			8, 10, 11, 12, 13, 15
<i>Lissoclinum fragile</i>	X		X	X				X		X		11, 13, 15
<i>Botryllus planus</i>			X	X		X						6, 13
<i>Botryllus tuberatus</i>	X		X	X			X	X	X			3, 8, 11, 13, 15
<i>Botrylloides nigrum</i>	X		X	X	X	X	X	X				4, 8, 11, 13, 14, 15
<i>Symplegma rubra</i>		X	X	X	X							8, 10, 11, 13, 14
<i>Styela canopus</i>			X	X		X		X	X			5, 11, 13
<i>Styela plicata</i>	X		X	X		X						1, 2, 3, 4, 5, 11, 13, 14
<i>Microcosmus exasperatus</i>	X	X	X	X	X	X	X	X				1, 2, 3, 4, 7, 10, 11, 13, 14, 15
<i>Molgula phytophila</i>	X	X		X								5, 12

1) van Name (1945), 2) Bjornberg (1956), 3) Millar (1958), 4) Rodrigues (1962), 5) Monniot (1969/70), 6) Costa (1969), 7) Millar (1977), 8) Rodrigues & Rocha (1993), 9) Rocha & Monniot (1995), 10) Rocha & Nasser (1998), 11) Rodrigues *et al.* (1998), 12) Rocha & Moreno (2000), 13) T. Lotufo, personal communication (2002), 14) Rocha & Costa (2005), 15) Rocha *et al.* (2005).

Distaplia bermudensis, *Cystodytes dellechiaiei*, *Didemnum granulatum*, *Lissoclinum fragile*, *Diplosoma listerianum*, *Perophora multiclathrata*, *Botrylloides nigrum*, *Botryllus tuberatus*, *Symplegma rubra*, *Styela canopus* and *Microcosmus exasperatus*. Of these, *Clavelina oblonga*, *Distaplia bermudensis* and *Symplegma rubra* also have disjunct distributions: southern and southeastern Brazil, as well as the Caribbean and North America (Figs 8-10, Tab. II). While this distribution is quite similar to that of clearly introduced species, the lack of information for these species causes us to tentatively call them cryptogenic. Though few studies have taken place on the northeastern Brazilian coast, these species are quite easily encountered and identified. If they occurred in that region, it is very likely that they would have been recorded. Thus, while probably introductions, it is difficult to determine in which direction the introductions went – from north to south, or vice versa. It is believed that they are native to the western Atlantic; the few encounters in other continents are considered introductions.

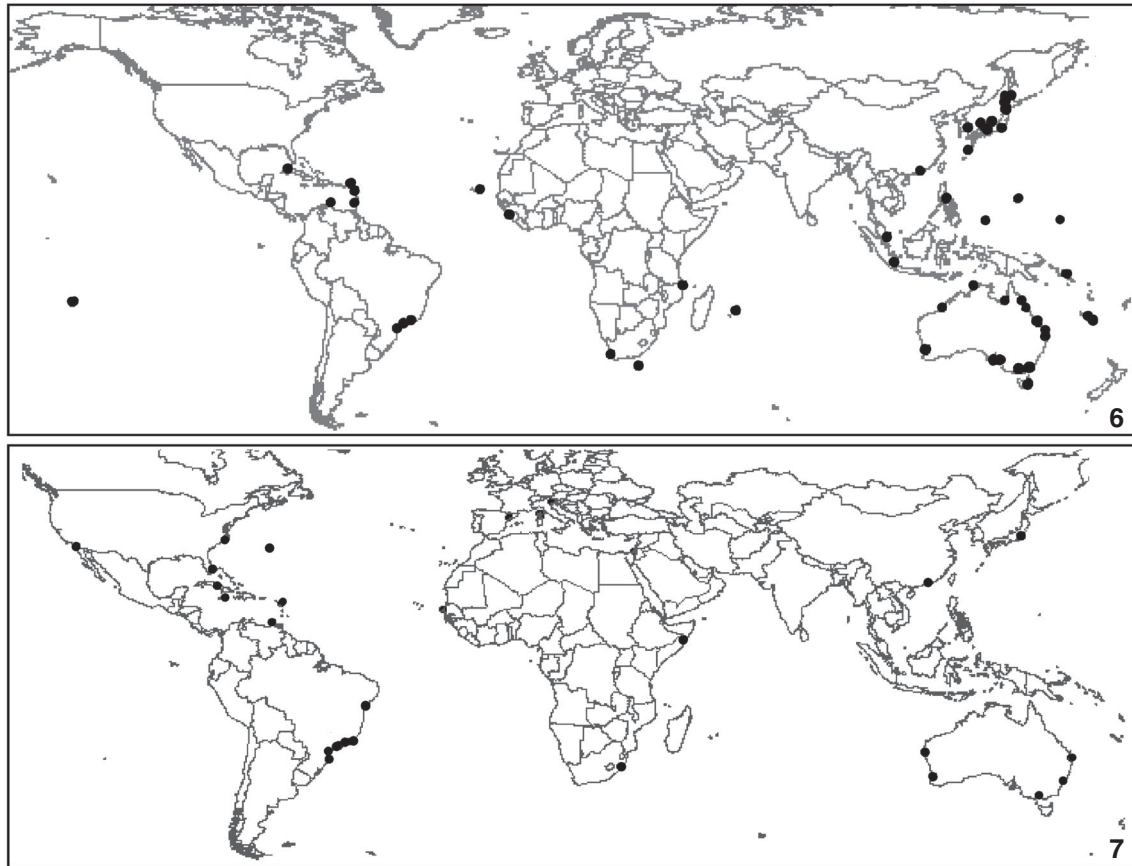
Clavelina oblonga, according to VAN NAME (1945) referring to the material Hartmeyer collected in São Sebastião, São Paulo, was first recorded in Brazil in ~1910. *Clavelina oblonga* is also found near Dakar, Senegal, and at Ilha Faial, Azores. In the Azores, it was introduced from American populations in 1971, and is restricted to Horta Harbor (MONNIOT & MONNIOT 1994).

The first record for *Distaplia bermudensis* in Brazil is that of MILLAR (1958), at Ubatuba, São Paulo. PÈRES (1957) recorded this species in the Mediterranean Sea (Balears Islands), but possibly erroneously identified. It was perhaps *Distaplia rosea* Della Valle, 1881, common in the Mediterranean. Since this species is only found on natural substrates it is probably native, and therefore should be carefully studied (substrate, location, etc.) in future Brazilian studies to clarify its status.

Symplegma rubra was first recorded in 1993 in São Sebastião, São Paulo, Brazil (RODRIGUES & ROCHA 1993). However, Rodrigues (unpublished data) encountered this species in the region since the 1960s.

The remaining cryptogenic species are widely distributed in the world and the entire Brazilian coast (the fourth distribution pattern; Figs 11-19, Tab. II). Thus it is difficult to determine the origin of these species and their points of introduction.

Perophora multiclathrata has been recorded three times in Brazil, the first of which was RODRIGUES *et al.* (1998). It is possibly distributed throughout the Brazilian coast, but being a cryptic species, is not easily found. It was found only once in the collections from Paranaguá Bay, along with *Botryllus planus*. LAMBERT (2003) also classified this species as cryptogenic in Guam.



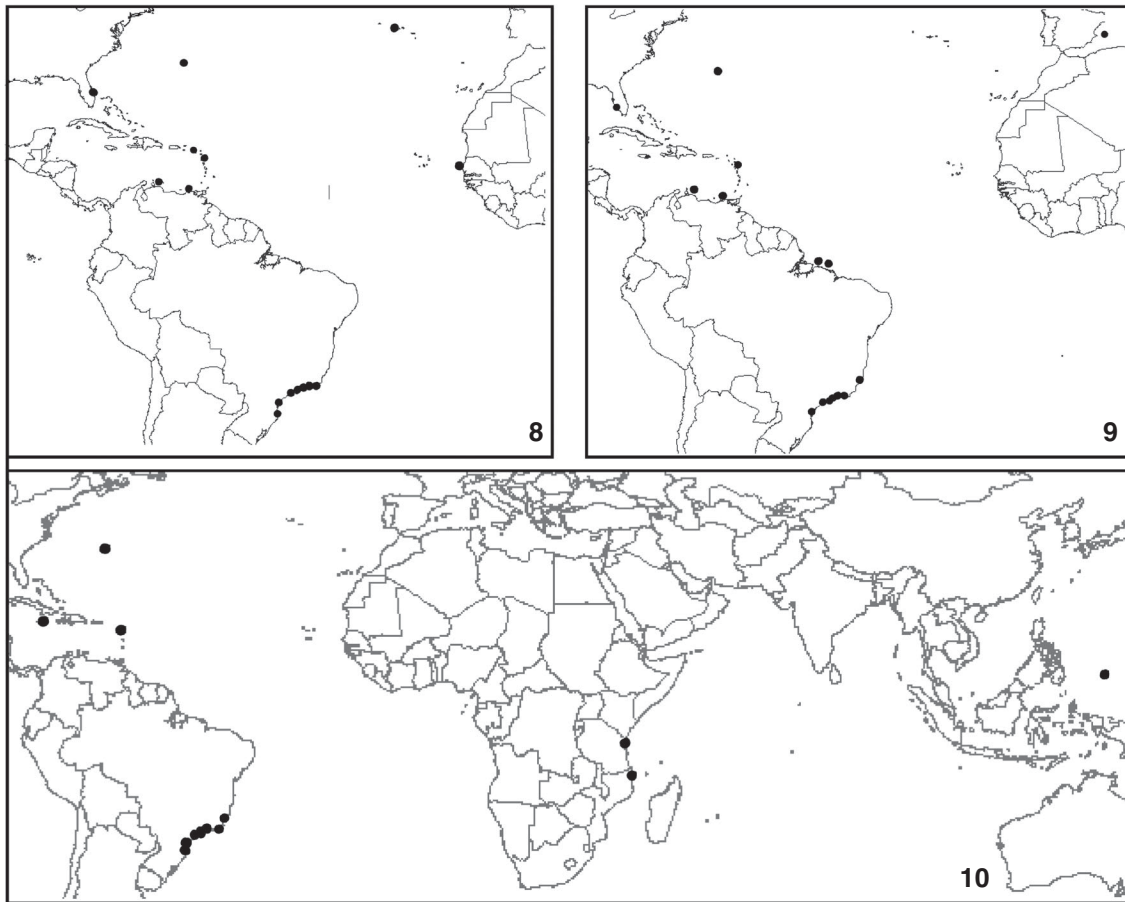
Figures 6-7. Distributions of the species found in Paranaguá Bay and nearby, that have widespread distributions and are disjunct in the western Atlantic: (6) *Ascidia sydneyensis*; (7) *Styela plicata*.

The first record for *Cystodytes dellechiaiei* on the Brazilian coast was HERDMAN (1886, in MILLAR 1958), probably in Bahia, with the name *C. draschii*, that today is considered synonymous with *C. dellechiaiei*. LAMBERT (2003) considered this a native species in Guam, because it was found predominantly on natural substrates. This is a difficult species to identify due to the extreme contraction of the zooids during fixation, and due to its relatively uniform morphology throughout its geographic distribution. In the Mediterranean, however, several morphotypes exist, most of which vary in color and spicule composition. Genetically, six clades are recognized, but which do not correspond with spicule shape and only partially with color (LOPEZ-LEGENTIL & TURON 2005). These results make it impossible to determine new species, but they indicate that *C. dellechiaiei* may not be just one, widespread, species as it is considered today.

Didemnum granulatum was found first in Brazil (São Paulo) in 1995 (ROCHA & MONNIOT 1995), and on the west African coast (Senegal) in 1994 (MONNIOT & MONNIOT 1994). This species has not been found in the Caribbean, nor on the eastern coast of Africa, and thus we believe it to be of Pacific origin.

Lissoclinum fragile was first recorded in Brazil by RODRIGUES *et al.* (1998) in São Paulo. The fact that it is found in ports, such as Apra Harbour, Guam (MONNIOT & MONNIOT 2001), Noumea port in New Caledonia (MONNIOT 1992), Malakal Harbor in Palau and also harbors in Honolulu, Hawaii (G. LAMBERT 2005, personal communication) suggests that it may be passively transported by ships. *L. fragile* is also considered introduced in Tahiti (MONNIOT *et al.* 1985) and was first reported on the west African coast in 1994 (MONNIOT & MONNIOT 1994).

Diplosoma from northeastern Brazil has been considered *Diplosoma macdonaldi* but this species is synonymous with *D. listerianum* (ROWE 1966) and the first record for Brazil was in the 1800s (HERDMAN 1886) in the state of Bahia. This species was first described from the English Channel by Milne-Edwards in 1841 but it is so widely distributed throughout the Atlantic, Indian and Pacific Oceans that it is difficult to define which is its actual native distribution. *Diplosoma listerianum* is recognized as introduced in Papeete, Tahiti (MONNIOT *et al.* 1985), Guam (LAMBERT 2002), and a historical record of observations and quantitative information for the last 25 years at a series of



Figures 8-10. Distributions of the species found in Paranaguá Bay and nearby, with disjunct distributions in the western Atlantic and some records outside the Atlantic: (8) *Clavelina oblonga*; (9) *Distaplia bermudensis*; (10) *Symplegma rubra*.

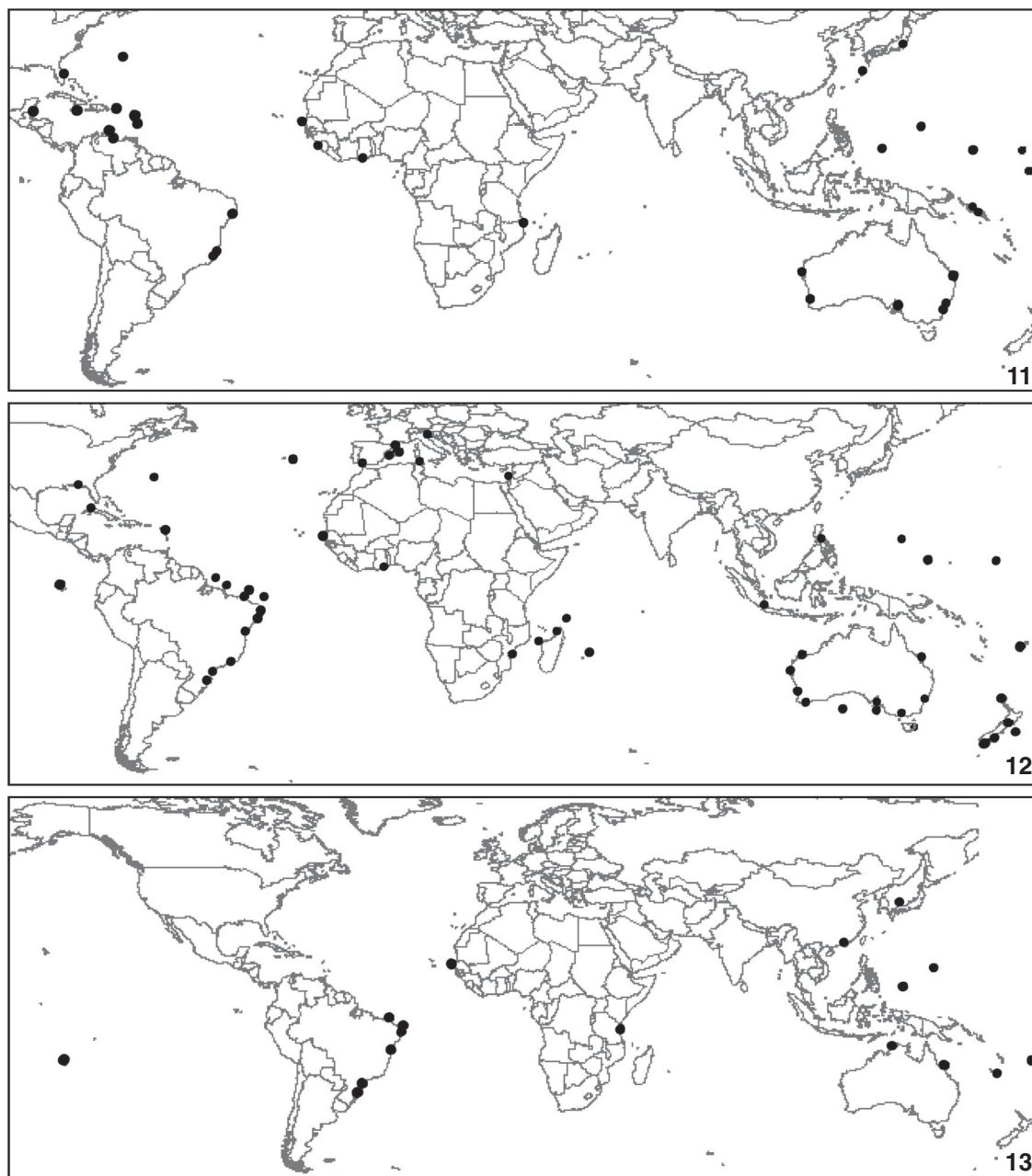
sites in the southwestern Gulf of Maine showed that it is a recent introduction there (HARRIS & TYRRELL 2001). This species forms chimera (an association of genetically different individuals within the same colony), and may store exogenous spermatozooids for several weeks. These characters favor the colonization of new areas (SOMMERFELDT & BISHOP 1999, LAMBERT 2001). Studies in São Paulo demonstrated other adaptations that favor introduction: recruitment throughout the year, great ability to colonize artificial substrates, rapid growth rates, and rapid sexual maturity (within one month; ROCHA 1991).

Botryllus tuberatus (under the name *B. primigenus* Oka, 1928) was first reported on the Brazilian coast in São Paulo and Rio de Janeiro (MILLAR 1958). This species is very different from the other botryllids by having only four rows of stigmata and it is likely that other species that also have four rows of stigmata have been designated as this species. Colonies vary in both form and color (MONNIOT 2002). KOTT (1985) considered its distribution unusual, since it is very rare for a species of the western Pacific. In both Paraná and Santa Catarina, large colo-

nies have been found, greater than 20 cm in diameter, and on natural vertical substrates. If this is an introduced species, it is quite successful in its colonization of natural environments. It is considered native in California where it occurs only in natural habitats on the open coast and never on artificial substrates (G. LAMBERT 2005, personal communication).

Botrylloides nigrum was first recorded in Brazil in the states of Santa Catarina and São Paulo (RODRIGUES 1962). In this study it was found on a cultivated mussel, and so possibly introduced. In Brazil, this species is usually epizooic, growing on algae and bivalve shells, such as oysters and mussels.

Styela canopus was first found at Ilha Grande, in Rio de Janeiro (MONNIOT 1969, 1970). Here, one sample was found on an abandoned fishnet on the ocean floor, suggesting that it was introduced. In São Sebastião, São Paulo, this species is found on artificial substrates used in scientific experiments (RMR, unpublished observation). MONNIOT (2002) described this species on wrecks on the coast near Djibouti, Africa. *Styela partita* (Stimpson, 1852) whose type location is Boston Harbor, in the

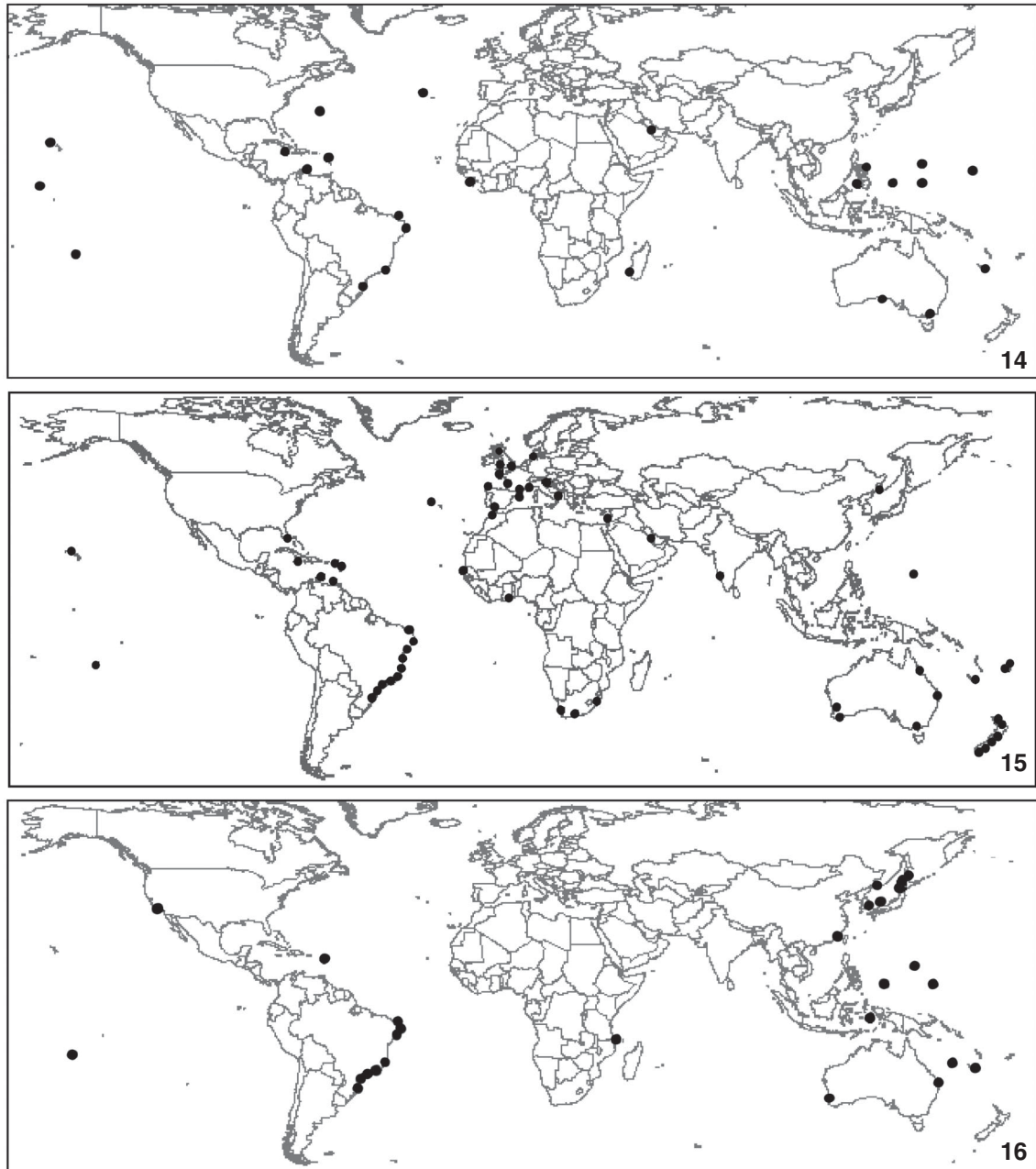


Figures 11-13. Distributions of the species found in Paranaguá Bay and nearby, with widespread and continuous distributions in the western Atlantic: (11) *Perophora multiclathrata*; (12) *Cystodytes dellechiaiei*; (13) *Didemnum granulatum*.

United States and shows an affinity for ports (VAN NAME 1945), was one of the species later synonymized with *S. canopus*. Thus, it is quite probable that *Styela canopus* is an introduced species in the western Atlantic and therefore southern Brazil.

Microcosmus exasperatus was first found in Brazil by VAN NAME (1945) at São Francisco do Sul, Santa Catarina. The type locality is in the Caribbean, but its original distribution is un-

known. Since many blanks exist in the distribution of this species, it is quite possible that it was introduced in a variety of places (MONNIOT 1981). Its first Mediterranean record was in 1963 in the military port Bizerte, and after this record many others were recorded, usually in ports (MONNIOT 1981), indicating its recent introduction here. MONNIOT (1965) recorded this species on the west coast of Africa in Pointe-Noire, Congo,

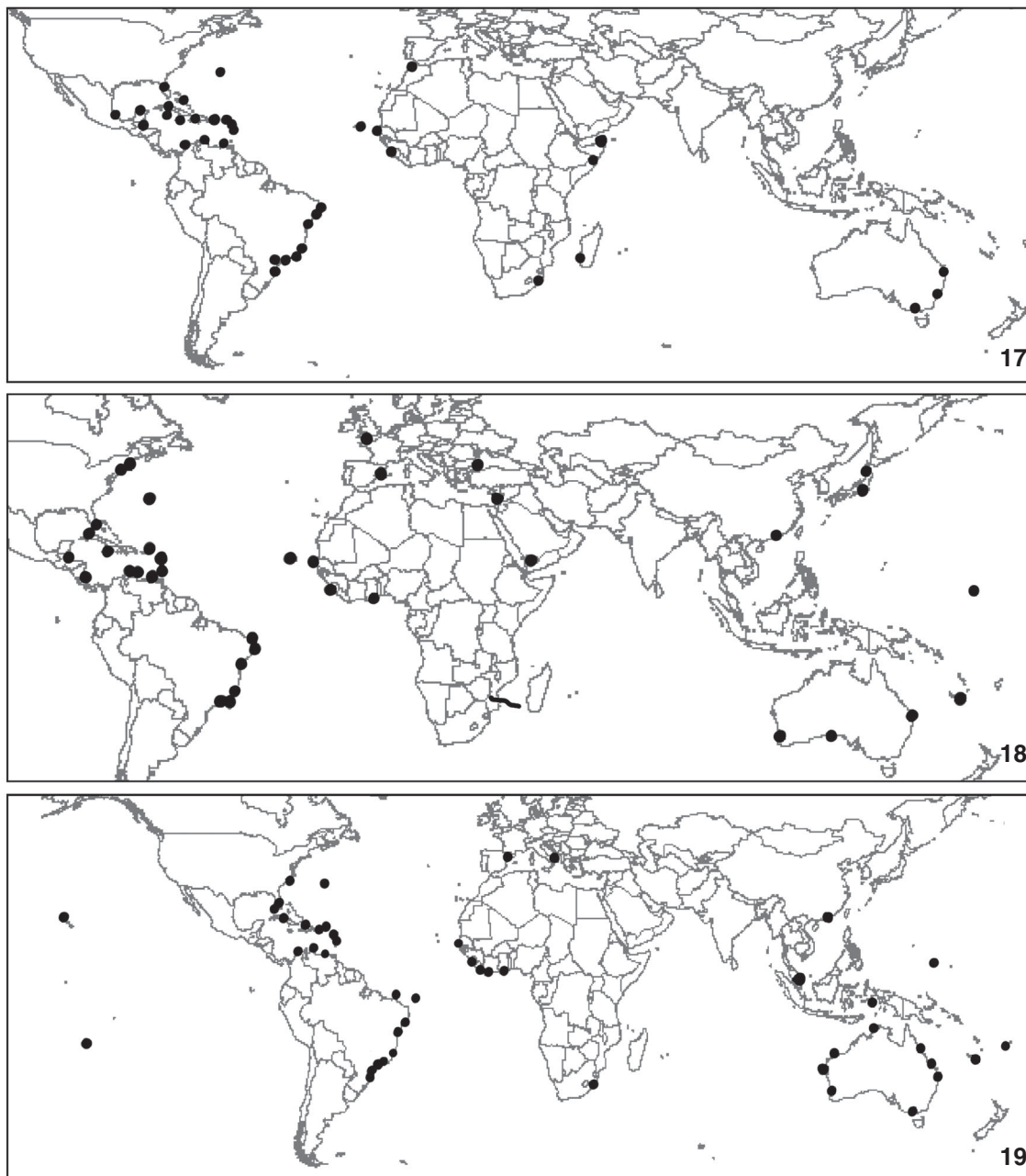


Figures 14-16. Distributions of the species found in Paranaguá Bay and nearby, with widespread and continuous distributions in the western Atlantic: (14) *Lissoclinum fragile*; (15) *Diplosoma listerianum*; (16) *Botryllus tuberatus*.

where rocky bottoms do not occur; it occurred only on the chains securing a floating dock, between 1–3 m depth. In New Caledonia, this species is abundant only on wharves and buoys at the Noumea Port (MONNIOT 1989).

Most species encountered in this study, with the exception of *Eudistoma carolinense* and *Distaplia bermudensis*, occur

on at least one type of artificial substrate, such as cultures, artificial reefs or the substrates common to ports (piers, buoys, columns and boats, etc.). The species *Ascidia curvata*, *A. sydneyensis*, *Botryllus planus* and *Clavelina oblonga* occur on oyster cultures (DALBY & YOUNG 1993, TOKIOKA 1952); *Cystodytes dellechiaiei* has been found on shrimp nets (MILLAR 1988);



Figures 17-19. Distributions of the species found in Paranaguá Bay and nearby, with widespread and continuous distributions in the western Atlantic: (17) *Botrylloides nigrum*; (18) *Styela canopus*; (19) *Microcosmus exasperatus*.

Diplosoma listerianum was found on fish hatchery structures at Bahrain, in the Arabian Gulf (MONNIOT & MONNIOT 1997). Cultures are very favorable for colonization by ascidians, since the substrates are simple and associated with ecological disturbances (LAMBERT 2001, 2005b). In at least one study, ascidians growing over cultured oysters did not influence the oyster growth rates

(DALBY & YOUNG 1993), yet in some circumstances the cover due to ascidians may be so great that they must exert a strong impact on the cultured animals.

Species' historical records are fundamental for determining introduction status. Unfortunately, only three studies prior to ours exist for ascidians in Paranaguá Bay region: MOURE *et al.*

(1954) recorded three species (*Didemnum candidum* Savigny, 1816; *Polysyncraton amethysteum* (van Name, 1902) and *Styela plicata*). Only the latter has been found again, with one individual on a mussel culture (*Perna perna* Linnaeus, 1758). This species is apparently not very successful in the region. *Didemnum candidum* is really a group of poorly identified species and which makes any interpretation difficult. While not found in our study, *Polysyncraton amethysteum* may be found in nearby Guaratuba Bay.

An experimental study of the encrusting community on artificial plates in Paranaguá Bay found *Clavelina oblonga*, *Didemnum speciosum*, *Diplosoma singulare* Lafargue, 1968, *Polyclinum constellatum* Savigny, 1816, *Styela plicata*, *Symplegma viride* Herdman, 1886 (probably *Symplegma brakenhielmi*) (M. D. CORREIA 1989, unpublished data). Of these, only *Clavelina oblonga* and *Styela plicata* were found in this study and were considered cryptogenic and introduced, respectively. *Clavelina oblonga* may actually be native, since it is abundant in all areas of the study.

The most recent study of ascidians in the bay includes the following species: Praia de Encantadas, Ilha do Mel: *Ascidia curvata*, *Ascidia sydneiensis*, *Clavelina oblonga*, *Didemnum granulatum*, *Didemnum lutarium* van Name, 1910, *Diplosoma listerianum*, *Distaplia bermudensis*, *Cystodytes dellechiaiei*, Praia de Fora, Ilha do Mel: *Symplegma rubra* Monniot C, 1972 and Ilha da Figueira: *Microcosmus exasperatus* (ROCHA & NASSER 1998). The differences between that study and this one may be due to two, not mutually exclusive reasons. First, neither study was intensive over the long term, and so it is not surprising that all existing species are not recorded in either of them. Second, some of the species may have been introduced since 1998. A comparison between the species found within the bay (Ilha das Cobras and Pier Tenenge) and on nearby islands (Ilha do Mel and Galheta), finds that *Perophora muticlathrata*, *Distaplia bermudensis*, *Lissoclinum fragile*, *Botryllus planus* and *Symplegma rubra* were found only within the bay, which strongly suggests introduction. On the other hand, *D. bermudensis* was once found on Ilha do Mel (ROCHA & NASSER 1998), *B. planus* is often found on rocky shores in Guaratuba, where *P. muticlathrata* has also been found. *P. muticlathrata*, however, is a cryptic species and will require a greater effort to be consistently found.

Comparing the ascidian fauna from Paranaguá Bay with that found in the more southern, isolated from ship traffic, Arvoredo Biological Reserve (ROCHA *et al.* 2005), of the 18 species reported here, 12 were found at the reserve: *Clavelina oblonga*, *Eudistoma carolinense*, *Cystodytes dellechiaiei*, *Distaplia bermudensis*, *Didemnum granulatum*, *Diplosoma listerianum*, *Lissoclinum fragile*, *Botrylloides nigrum*, *Botryllus tuberatus*, *Botryllus planus*, *Styela canopus* and *Microcosmus exasperatus*. Since many of these species are considered cryptogenic or introduced in southern Brazil, a monitoring program of the reserve could be very informative to understand the population dynamics of these species, and any potential problems associated with their introductions.

Among the ascidian species that are the most common introduced species worldwide, four were found in Paranaguá Bay: *Diplosoma listerianum*, *Styela canopus*, *Styela plicata* and *Microcosmus exasperatus*. This indicates that the Paranaguá Port is open to invasion of exotic species, and should be thoroughly monitored to understand the growth and recruitment of introduced populations, and to understand how to control those populations if it is deemed necessary. Since more groups of organisms besides ascidians, including many benthic invertebrates, are probably involved in these introductions, we urgently recommend a much needed larger scale inventory of the fauna potentially influenced by port activities. Only with such an inventory will we be prepared to undertake the necessary measures should we discover potentially harmful and economically costly introduced organisms.

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