Journal of the Marine Biological Association of the United Kingdom, 2013, 93(1), 273–282.
doi:10.1017/S002531541200063X

Fifty years of ascidian biodiversity research in São Sebastião, Brazil

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The city of São Sebastião (SS), in south-eastern Brazil, is one of the hotspots for marine research since the establishment of the Centre of Marine Biology of the University of São Paulo in the 1960s. The SS region experienced intense transformation during the past 50 years, including increasing urbanization and construction of maritime facilities. Ascidian surveys during the past 50 years have found 62 species, eight of which were described as new and 12 are introduced. Didemnidae and Styelidae are the most speciose families in São Sebastião Channel, with 20 and 15 species respectively. Phallusia nigra, Didemnum psammatodes, Trididemnum orbiculatum, Botrylloides nigrum *and* Symplegma rubra *are the most common ascidians. Most of the species are of tropical origin and São Paulo is their southern geographical limit. Comparisons of reports of the ascidians from different time periods allowed detection of species introductions and shifts in assemblage structure in terms of both species composition and abundance. Additionally, we discuss the main taxonomic issues regarding ascidians from south-eastern Brazil and identify profitable areas for future research. We believe that the temporal data compiled here will serve as a baseline for monitoring and management of ascidians in SS. Additionally, this study provides one of the most detailed datasets of ascidian diversity from the south-western Atlantic Ocean.*

Keywords: Tunicata, Ascidiacea, encrusting communities, hard substrate

Submitted 12 November 2011; accepted 13 April 2012; first published online 7 June 2012

INTRODUCTION

Ascidians are among the dominant groups of benthic invertebrates in many marine sessile communities, on both natural and artificial substrates (Jackson, 1977). Solitary and colonial ascidians are frequently seen covering large portions of rocky substrates in the intertidal and shallow subtidal zones, especially in shaded areas (Lambert, 2005). Coastal areas are frequently exposed to intense perturbations due to human colonization and consequent urbanization; as a result, ascidians are exposed to diverse anthropogenic disturbances that may reduce their abundance and diversity (Naranjo *et al.*, 1996). In contrast, local increase in the number of species is apparently the result of modifications of the marine environment, affecting the sessile community organization, and facilitating the invasion of exotic ascidians from around the world (Chapman & Blockley, 2009). Human-mediated reductions or increases in ascidian abundances and diversity are both potentially threatening to sessile communities, because they may reshape the trophic structure of marine communities (Byrnes *et al.*, 2007; Stachowicz *et al.*, 2007), and also to the human economy, especially associated with impacts on mariculture (McKindsey *et al.*, 2007).

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In Brazil, the human population is mainly in coastal areas or near coastal cities, all along its 8500 km shoreline. São Sebastião (SS) is one such city, located on the south-eastern coast (Figure 1), about 180 km from Brazil's largest city, São Paulo. The coastal area near SS has alternating rocky shores and sandy beaches, with many coastal islands. The climate is subtropical, and water temperatures range from 12°C at the bottom (50 m deep) to 28°C on the surface during the summer months (Silva *et al.*, 2005). The most populated area is confined between the hills of the Serra do Mar and the São Sebastião Channel (SSC), a passage formed between the continent and São Sebastião Island, the largest coastal island in the region. Channel waters have a maximum depth of 47 m and are relatively well-protected, which provided the motive for the construction of an oil terminal, a harbour and a few marinas.

Since the 1950s this region has undergone severe changes, for the most part as a result of urbanization. For example, the São Sebastião Harbour was officially inaugurated in 1955 and another terminal for oil and gas began operation in 1969 (http://www.portodesaosebastiao.com.br). The busy traffic of international ships during the past 40 years greatly increased the probability of introduction of alien species in the region. Today, almost 70,000 people live in SS, with more than double that number during summer vacations, while on the other side of the SSC, the population of Ilhabela city increases ten-fold during the summer (CETESB, 2009). The intense degradation of the hills along the coast has also caused an increasing amount of erosion, turning the water almost

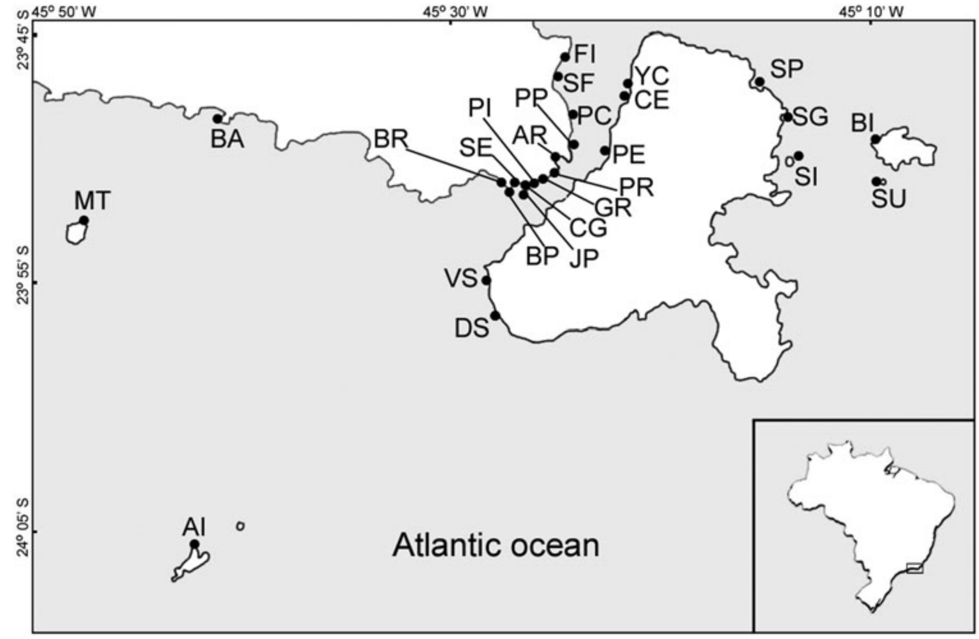

Fig. 1. Map of the São Sebastião channel on the south-eastern Brazil, showing localities from where data were compiled, where: AI, Alcatrazes Island; AR, Araçá Beach; BA, Baleia Beach; BP, Baleeiro Point; BR, Barequeçaba Beach; BI, Búzios Island; CE, São Sebastião Island (Centro Beach); CG, Cabelo Gordo Beach; DS, São Sebastião Island (Dart shipwreck); FI, Figueira Beach; GR, Grande Beach; JP, Jarobá Point; MT, Montão de Trigo Island; PC, Pontal da Cruz Pier; PE, São Sebastião Island (Perequê Beach); PI, Pitangueiras Beach; PP, Petrobras Pier; PR, Preta Beach; SE, Segredo Beach; SF, São Francisco Beach; SG, São Sebastião Island (Saco da Ponta Grossa); SI, Serraria Island; SP, São Sebastião Island (Saco do Poço); SU, Sumítica Island; VS, São Sebastião Island (Velasquez shipwreck); YC, São Sebastião Island (Yatch Club).

permanently murky. It is reasonable to expect that the arrival of alien species with the incoming ships and the continuous degradation of coastal habitats might have caused important shifts in the marine fauna and flora.

São Sebastião city is also home to the Center of Marine Biology (CEBIMar) of the University of São Paulo. Since 1962 when it was incorporated within the university, this research institute resulted in very important contributions to the knowledge about the local marine biota. Prior to the work of Rodrigues (1962, 1966, 1977) on Brazilian ascidians, only a few records for the region were noted by Van Name (1945), based on material collected by Lüderwaldt (1929). Later, Millar (1958) also published on Brazilian ascidians, including material from SS. During the 1990s, a series of papers on the biodiversity and taxonomic issues of ascidians was published (Rocha, 1988, 1991; Rocha & Monniot, 1993, 1995; Rodrigues & Rocha, 1993), culminating with the production of the first identification guide for Brazilian ascidians (Rodrigues *et al.*, 1998). By the end of that decade, an important research programme was created, with the ambitious goal of describing the biodiversity of the State of São Paulo. The BIOTA programme compiled a first series of reports of up-to-date data on known diversity for many taxa, including ascidians (Rodrigues *et al.*, 1999), and a new series of papers provided additional information (Dias & Rodrigues, 2004; Dias *et al.*, 2006, 2008, 2009; Lotufo & Dias, 2007; Bonnet & Rocha, 2011; Rocha *et al.*, 2011).

As the SSC is threatened by the future expansion of the São Sebastião Harbour, and because an important body of information on diversity of ascidians from Brazil is not accessible to English-speaking researchers, the purpose of this paper is to summarize the knowledge about the diversity of ascidians from south-eastern Brazil, describing the main shifts in species compositions and dominance of ascidians over the past 50 years, in order to establish a baseline for future monitoring. We also discuss the main taxonomic issues regarding ascidians from south-eastern Brazilian waters and identify profitable areas for future research. Additionally, this study provides one of the most detailed datasets about ascidian diversity from the south-western Atlantic Ocean.

MATERIALS AND METHODS

The list of species and localities was compiled from published data and from material collected by the authors, including unpublished data. Ascidian diversity was studied in 26 localities between 23°45′S 45°50′W and 24°05′S 45°10′W (Figure 1). Vouchers specimens are available at the Museu de Zoologia–USP and the Ascidiacea collection of the Zoology Department, Universidade Federal do Paraná (http://splink.cria.org.br/manager/detail?resource=DZUP-Ascidiacea&setlang=pt&system=–Specieslink).

Abundance was estimated from 1980 to 1985 by reviewing survey studies and description of species published for the studied area. After 1985, abundance was estimated based on observational information by the authors while in the field. In this review, we standardized the relative abundance of the species, considering it *rare* if not seen in all field trips and when found was represented by no more than one or two colonies/individuals, *uncommon* if seen in all field trips but in very low abundance, *common* if seen in all field trips and abundant enough to be found without searching for it, and *very common* if it was a conspicuous species dominating the encrusting community either on natural or artificial substrate.

Studies reviewed here included surveys at natural substrata in the intertidal zone (Van Name, 1945; Millar, 1958; Rodrigues 1962, 1966, 1977; Dias *et al.*, 2009), the subtidal

https://doi.org/10.1017/S002531541200063X Published online by Cambridge University Press

zone (Rocha *et al.*, 1999, 1996, 2002, 2008, 2009 unpublished data), experimental studies using artificial panels (Rocha, 1988; Dias *et al.*, 2008) and experimental studies on intertidal boulder communities (Rocha, 1991). Most of the observations before 1990 were in the intertidal zone or from submerged experimental panels, supplemented within the past 20 years by SCUBA diving surveys. The surveys were more frequent in the years 1960–1970, 1985–1992, 1994–1996 (SCUBA diving included), 1999–2002 (SCUBA diving included) and 2008–2009 (SCUBA diving included) when different research projects involving ascidians were taking place.

RESULTS

As a result of the almost continuous record of the ascidian fauna for the past 50 years, with only six species recorded for SS in 1945 (Van Name, 1945), a total of 62 species are now known (Figure 2; Table 1). The type locality of eight of these species is the SS region. Among the 12 different families represented by these 62 species, Didemnidae (20 species) and Styelidae (15 species) were the most speciose in the SSC, while the families Clavelinidae, Euherdmaniidae and Cionidae were the least speciose, with only a single species each (Table 1). Thirty per cent (19) of the species are solitary, most of which are rare (12), with fewer than 10 individuals found in the past 50 years, or uncommon (3). Most species are tropical or typically found in warm subtropical waters. Exceptions are the introduced temperate-water species *Lissoclinum perforatum* and *Ciona intestinalis*. No species has a Patagonian or Magellanic distribution.

Only 18 species can be definitely considered native to this region, while another 12 are introduced, 29 are cryptogenic and 3 were not classified. Although classified as cryptogenic (Table 1), due to the lack of information about their geographical origin, the species *Cystodytes dellechiajei*, *Ascidia curvata*, *A. interrupta*, *A.* cf. *multitentaculata*, *A. tenue*, *Polycarpa spongiabilis*, *Polyandrocarpa anguinea, Symplegma rubra* and *Pyura vittata* have isolated and small populations

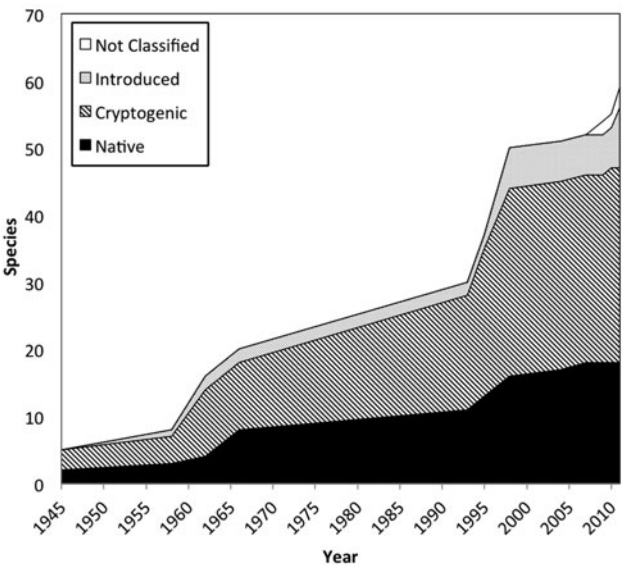

Fig. 2. Cumulative number of native, cryptogenic and introduced species of ascidians recorded from São Sebastião, Brazil, from 1945 to present.

and their southernmost geographical limit is in SS or a little further south, suggesting that their presence is due to human transportation. Another group, comprising *Polyclinum constellatum*, *Didemnum perlucidum*, *Lissoclinum fragile*, *Diplosoma listerianum*, *Styela canopus*, *Microcosmus exasperatus*, *Herdmania pallida* and *Pyura vittata* has a more widespread distribution, is very common in many ports around the world, and is likely to have been introduced many years ago, probably by navigation in the 16th Century. Collections made by the authors near CEBIMar showed that many species arrived but did not persist in the new environment. One such species was *Distaplia* sp., common during the 1990s but not retrieved on surveys made in 2008 and 2009. It is worth mentioning also that many species were not even identified, because they were observed only once or twice in subsequent months and they were mostly juveniles, without mature gonads or larvae for complete identification (these species are not listed in Table 1).

The overall abundance of the species registered since the 1980s has not markedly changed since then (Table 2). Nonetheless, some species were only recently found, due to the relatively recent use of SCUBA to explore the subtidal region (i.e. *Ascidia multitentaculata*, *Perophora* sp., *Cystodytes dellechiajei*, *Didemnum granulatum*, *D. ligulum*, *D. rodriguesi* and *Lissoclinum perforatum*) or because they are very recent introductions (i.e. *Distaplia stylifera*, *Distaplia* sp., *Eusynstyela* sp., *Cnemidocarpa irene* and *Aplidiopsis* sp.). Also, two species declined in abundance: *Polysyncraton* sp. (previously identified as *P. amethysteum*: see the section about taxonomic issues for details), was reasonably abundant in the low intertidal zone before 2000, after which it declined severely; *Didemnum rodriguesi* type material was collected in the intertidal zone of Grande Beach, where the species was common. That species then declined precipitously, and between 2001 and 2002, one of us (G.M. Dias, unpublished data) monthly sampled the type locality searching for ascidians on boulders located within 15 randomly selected 1 m^2 quadrats and not a single specimen was found. However, it was seen in the subtidal zone elsewhere on São Sebastião Island in December 2008 (region of the wrecks 'Velasquez' and 'Dart'), Búzios Island, Sumítica Island and Serraria Island.

In the intertidal zone, exposed vertical surfaces of boulders are dominated by *Didemnum psammatodes*, while the undersurface of rocks usually harbours a large diversity of didemnid and styelid colonial species, with no single, dominant, species on large areas of rock. In the shallow subtidal zone, *Phallusia nigra*, *Trididemnum orbiculatum*, *Botrylloides nigrum* and *Symplegma rubra* are the dominant species on the exposed surface of the rocks (Table 2). The solitary *P. nigra* is probably the most conspicuous ascidian in SS, because it is very abundant and its black colour contrasts against the rocky background. The other two common solitary species are *Microcosmus exasperatus* and *Herdmania pallida*, both less abundant and usually camouflaged by epibionts.

Fouling communities associated with artificial substrates (experimental panels) are usually dominated by *Diplosoma listerianum*, *Didemnum perlucidum*, *Symplegma* spp., *Botrylloides nigrum*, *Botrylloides giganteum*, *Clavelina oblonga*, *Polyclinum constellatum* and *Styela canopus* (Table 2). All these species, with the exception of *B. giganteum*, have been abundant in the region for at least the past 30 years.

https://doi.org/10.1017/S002531541200063X Published online by Cambridge University Press

Table 1. List of species from the São Sebastião region and introduction status (C, cryptogenic; N, native; I, introduced; ?, no information available).

Species	Status	Locality[1]	Habitat[2]	References[3]
Clavelinidae				
Clavelina oblonga (Herdman, 1880)	C	Abundant in the whole São Sebastião Channel	N, A, SS	1, 2, 3, 4, 7, 10
Polycitoridae				
Cystodytes dellechiajei (Della Valle, 1877)	C	São Sebastião Island	NR, SS	10
Eudistoma clavatum (Rocha & Bonnet, 2009)	N*	AI	NR, SS	14
Holozoidae				
Distaplia bermudensis (Van Name, 1902)	N	BI, CE, GR, MT, PR, SE, SP, YC,	NR, A, I, SS	3, 7, 10
Distaplia stylifera (Kowalewsky, 1874)	I	PP, YC	A, SS	This study
Distaplia sp.	I	JP	NR, SS	10
Polyclinidae				
Aplidiopsis sp.	I	YC	A, SS	This study
Aplidium accarense (Millar, 1953)	C	BI, CG, GR, PC, PP, PR, SE, SI, YC	NR, I, SS	10, 14
Polyclinum constellatum (Savigny, 1816)	C	AR, BA, BR, CE, GR, PC, PR, YC	NR, A, SS	2, 3, 4, 7, 10
Euherdmaniidae				
Euherdmania vitrea (Millar, 1961)	N*	BR	NR, SS	10
Didemnidae				
Didemnum ahu (Monniot & Monniot, 1987)	C	CG, GR, BP	NR, I	9, 10
Didemnum apersum (Tokioka, 1953)	I	GR	NR, I	11
Didemnum cineraceum (Sluiter, 1898)	I	AI, CG, PI, PP, PR, SE	NR, A, I, SS	10, 14
Didemnum galacteum (Lotufo & Dias, 2007)	N*	BA, BP, GR, JP, PR	NR, I, SS	9, 10, 13
Didemnum granulatum (Tokioka, 1954)	C	BA, BI, BP, CG, DS, GR, JP, PR, SU	NR, I, SS	9, 10, 14
Didemnum ligulum (Monniot, 1983)	C	BP, CG, GR, JP, SI, PR, São Sebastião Island	NR, I, SS	9, 10
Didemnum perlucidum (Monniot, 1983)	C	BI, BP, CE, CG, PR, VS, YC	NR, A, I, SS	9, 10
Didemnum psammatodes (Sluiter, 1895)	C	AR, BR, DS, FI, GR, PC, PE, PR, SE	NR, I	7, 9, 10
Didemnum rodriguesi (Rocha & Monniot, 1993)	C*	BI, BP, BR, GR, SU, VS	NR, I, SS	8, 10, 14
Didemnum speciosum (Herdman, 1886)	N	AR, BP, CG, GR, JP, PR	NR, I, SS	1, 7, 10
Didemnum tetrahedrum (Dias & Rodrigues, 2004)	N*	BA	NR, I	12
Didemnum vanderhorsti (Van Name, 1924)	N	BA, BI, BP, CG, JP, PR, SI, São Sebastião Island	NR, I, SS	7, 10
Diplosoma listerianum (Milne-Edwards, 1841)	C	AR, BA, GR, PR, SE	NR, A, I, SS	1, 7, 10, 14
Lissoclinum fragile (Van Name, 1902)	C	BP	NR, A, I, SS	10, 14
Lissoclinum perforatum (Giard, 1878)	I	GR, AI, São Sebastião Island	NR, SS	10, 14
Lissoclinum sp.	?	BP	NR	10
Polysyncraton sp. (Van Name, 1902)	N	BA, BR, GR, PR, SE, São Sebastião Island	NR, I, SS	2, 3, 4, 7, 10, 14
Trididemnum orbiculatum (Van Name, 1902)	N	BA, BR, CE, GR, PP, PR, SE	NR, I, SS	7, 10, 14
Trididemnum sp. 1	N	GR, SE	NR, A	15
Trididemnum sp. 2	?	DS, VS	NR, A, SS	This study
Cionidae				
Ciona intestinalis (Linnaeus, 1767)	I	AI, SE, SF	NR, A, I, SS	2, 3, 10, 14
Perophoridae				
Perophora multiclathrata (Sluiter, 1904)	C	BA, SE, SF	NR, A, I, SS	10
Perophora viridis (Verrill, 1871)	C	AI, BI, GR, PR, SE	NR, A, I, SS	10, 14
Perophora sp.	?	BI	NR	This study
Ascidiidae				
Ascidia curvata (Traustedt, 1882)	C	CG, SE	NR, A, I, SS	10
Ascidia interrupta (Heller, 1878)	C	CG, SE	NR, A, I, SS	1, 10
Ascidia cf. *multitentaculata* (Hartmeyer, 1912)	C	SE, YC	A	This study
Ascidia sydneiensis (Stimpson, 1855)	I	AR, PC, SE	NR, A, I, SS	2, 3, 4, 10
Ascidia tenue (Monniot, 1983)	C	BP, JP	NR, ST	16
Phallusia nigra (Savigny, 1816)	C	Abundant in the whole São Sebastião Channel	NR, SS	1, 2, 3, 4, 10
Styelidae				
Botrylloides giganteum (Pérès, 1949)	C	BA, CG, GR, PC, PR, SE, YC	NR, A, SS	7, 10
Botrylloides nigrum (Herdman, 1886)	C	AR, CG, GR, PC, PR, SE	NR, A, I	7, 10
Botryllus tabori (Rodrigues, 1962)	N*	AR, BA, GR, PR, SE	NR, I	4, 7, 10
Botryllus tuberatus (Ritter & Forsyth, 1917)	C	BA, GR, PR, SE	NR, A, I, SS	3, 7, 10
Cnemidocarpa irene (Hartmeyer, 1906)	I	SU	NR, SS	This study
Eusynstyela tincta (Van Name, 1902)	C	BA, GR, JP, PR, SF	NR, SS	10
Eusynstyela sp. (Van Name, 1921)	I	BR, CE, PC, PP, SE, SP	NR, A, SS	10, 11
Polyandrocarpa anguinea (Sluiter, 1898)	N	BR, JP, São Sebastião Island	NR, A, SS	1, 3, 4, 6, 10
Polyandrocarpa zorritensis (Van Name, 1935)	C	AR, BA, PC, SE, São Sebastião Island	NR, A, SS	2, 3, 10
Polycarpa spongiabilis (Traustedt, 1883)	I	São Sebastião Island	NR, SS	10
Styela canopus (Savigny, 1816)	C	CG, SE, PC, YC	A, SS	10, 14
Styela glans (Herdman, 1881)	N	Channel bottom	NS	10
Styela plicata (Lesueur, 1823)	I	AR, CG, PI, YC	NR, A, SS	2, 4, 10
Symplegma brakenhielmi (Michaelsen, 1904)	C	AR, BA, CG, GR, PC, PR, SE, YC	NR, A, I, SS	2, 3, 4, 7, 10

Continued

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Table 1. Continued

Species	Status	Locality[1]	Habitat[2]	References[3]
Symplegma rubra (Monniot, 1972)	N	BA, CG, GR, PC, PE, PP, PR, SE, YC	NR, A, I, SS	7, 10
Pyuridae				
Herdmania pallida (Heller, 1878)	C	Abundant in the whole São Sebastião Channel	NR, A, SS	2, 3, 10
Microcosmus exasperatus (Heller, 1878)	C	GR, SE, YC	NR, SS	2, 3, 10
Pyura mariscata (Rodrigues, 1966)	N*	Channel bottom	NS	5, 10
Pyura millari (Rodrigues, 1966)	N*	Channel bottom	NS	5, 10
Pyura vittata (Stimpson, 1852)	C	AI, BI, SG, SI, SU, VS	NR, SS	5, 10, 14
Molgulidae				
Molgula braziliensis (Millar, 1958)	N	?	NR, I	3, 10
Paraeugyrioides vannamei (Monniot, 1970)	N	Channel bottom	NS, SS	10

*designates that SS region is the type locality of the species; [1]see legend of Figure 1 for localities codes; [2]NR, natural rock substrate; NS, natural soft substrate; A, artificial substrate; I, intertidal; SS, shallow subtidal (<20 m); [3]1, Van Name, 1945; 2, Bjornberg, 1956; 3, Millar, 1958; 4, Rodrigues, 1962; 5, Rodrigues 1966; 6, Rodrigues, 1977; 7, Rodrigues & Rocha, 1993; 8, Rocha & Monniot, 1993; 9, Rocha & Monniot, 1995; 10, Rodrigues *et al.*, 1998; 11, Lotufo, 2002; 12, Dias & Rodrigues, 2004; 13, Lotufo & Dias, 2007; 14, Rocha & Bonnet, 2009; 15, Dias *et al.*, 2008; 16, Bonnet & Rocha, 2011.

DISCUSSION

This review shows that, with 62 species, the SS region is a hotspot of ascidian diversity in Brazil, in comparison with species richness in other, well-studied, Brazilian states: Santa Catarina—39 (Rocha *et al.*, 2005b, 2009); Paraná—32 (Rocha & Nasser, 1998; Rocha & Faria, 2005; Rocha & Kremer, 2005); Rio de Janeiro—47 (Millar, 1958; Simões, 1981; Lotufo, 2002; Rocha & Costa, 2005; Marins *et al.*, 2010); and Ceará—33 (Lotufo, 2002; Lotufo & Silva, 2006; Oliveira-Filho, 2010). Species richness for ascidians in the SSC is also comparable to that in False Bay (58 species), the richest site in South Africa (Awad *et al.*, 2002) and Bocas del Toro, Panamá (58 species), considered a very diverse region in the Caribbean (Rocha *et al.*, 2005a). Ascidian richness from SS comprises more than half of all the ascidian species known from Brazil (Rodrigues *et al.*, 1999) and 94% of the species of the State of São Paulo (Rocha *et al.*, 2011). Today, the SSC is apparently a region of high diversity for many groups, as shown by a compilation of a total of 733 species of invertebrates and algae in just one bay (Araçá Bay) within the channel (Amaral *et al.*, 2010).

Three important details contribute to these results. First, the many tunicate specialists working in the region resulted in a sampling effort (both temporally and spatially) that is many times greater than any other region in the country. Second, the region comprises very diverse habitats, including rocky shores, mangroves, rocky and sandy beaches and many islands within a relatively small area. Third, São Sebastião Harbour with international shipping and marinas accounts for the large number of introduced and cryptogenic species. Small boat traffic between SSC and other regions of Brazil can provide an important vector for increasing distribution of non-native species that have been introduced by other vectors such as sea chests of large ships (Wasson *et al.*, 2001). While some potentially invasive species were observed only once or twice in the proximities of the São Sebastião Harbour, many species persisted and are now established at least on artificial substrates (e.g. *Didemnum cineraceum*, *Eusynstyela* sp. and *Styela plicata*), suggesting that the region has favourable environmental conditions for ascidians.

Changes in the ascidian assemblage have been reported from different locations in the Atlantic. In Jamaica, Goodbody (1993) found important shifts in the ascidian assemblage dwelling on mangrove roots after 30 years of monitoring, with eight species disappearing, four clearly declining in abundance and two new arrivals, for a total of 16 from the initial 22 species. In the east Atlantic, examination of samples from the Azores, Cape Verde, Canary Islands, Sierra Leone and Senegal in the 1990s found 12 new records, mainly of cosmopolitan species commonly found in the western Atlantic or Mediterranean (Monniot & Monniot, 1994). These observations, with results described here, point to the need for more detailed monitoring of faunal shifts, as changes may happen over a temporal scale of a few years to decades.

As is the case for many marine invertebrate taxa, identification of ascidian species is often challenging, and taxonomic difficulties in some SSC species persist. Indeed, most taxonomic issues comprise didemnids, partly because of the reduced size of the zooids and comparatively few distinguishing characters. The complex taxonomic identity of the *Trididemnum* genus in SSC is a good example of this problem. *Trididemnum orbiculatum* has two morphotypes (intertidal and subtidal) that were considered by Monniot (1983) as synonyms. However, Dias *et al.* (2009) showed that the two morphotypes of *T. orbiculatum* are distinct species (using both genetic and morphological criteria). Because the subtidal morphotype corresponds to the original description by Van Name (1902), Dias *et al.* (2009) suggested that only this morphotype should keep the name *T. orbiculatum*, while the intertidal morphotype may be one or two species.

The only species of *Polysyncraton* in the SSC was originally identified as *Polysyncraton amethysteum* by Moure *et al.* (1954) and Millar (1958) based only on the description published by Van Name (1945). Recent analysis of the holotype (AMNH 1304) by Lotufo (2002) revealed important differences in the SS specimens. The holotype has larger systems, with a single cloacal opening on the colony, a unique arrangement of the spicules on the tunic and larger larvae, and so the Brazilian animals should be described as a new species. The genus *Diplosoma*, especially *Diplosoma listerianum*, also needs revision. Rowe (1966) reviewed the genus and found that *D. macdonaldi* and *D. rayneri* are junior synonyms of *D. listerianum*. Subsequently, Lafargue (1968) discussed the status of

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Table 2. Relative abundance of ascidians in the São Sebastião Channel. ?, without information; –, not observed; X, rare species; XX, uncommon species; XXX, common species; XXXX, very common species, frequently observed dominating the encrusting community.

Species	<1980	1980 to 1990	1990 to 2000	2000 to 2010	Observations
Clavelina oblonga	XX	XXX	XXX	XXX	Important seasonal variation
Cystodytes dellechiajei	?	–	–	X	
Eudistoma clavatum	–	–	–	X	
Distaplia bermudensis	?	XXX	XXX	XXX	
Distaplia stylifera	–	–	–	X	Only on artificial substrate
Distaplia sp.	–	–	X	–	Only on artificial substrate
Aplidiopsis sp.	–	–	–	X	Only on artificial substrate
Aplidium accarense	?	?	XX	XX	
Polyclinum constellatum	XX	XXX	XXX	XXX	
Euherdmania vitrea	X	X	X	X	
Didemnum ahu	?	X	X	X	
Didemnum apersum	–	–	X	X	
Didemnum cineraceum	?	?	X	XX	Only on artificial substrate
Didemnum galacteum	?	XXX	XXX	XXX	
Didemnum granulatum	?	XXX	XXX	XXX	
Didemnum ligulum	?	XXX	XXX	XXX	
Didemnum perlucidum	?	XXX	XXXX	XXX	More abundant on artificial substrate
Didemnum psammatodes	?	XXXX	XXXX	XXXX	
Didemnum rodriguesi	?	XX	XX	XXX	
Didemnum speciosum	?	XX	XX	XX	
Didemnum tetrahedrum	?	?	?	X	
Didemnum vanderhorsti	?	XXX	XXX	XXX	
Diplosoma listerianum	?	XXX	XXX	XXX	More abundant on artificial substrate
Lissoclinum fragile	?	XXX	XXX	XXX	More abundant on artificial substrate
Lissoclinum perforatum	?	?	XX	XX	
Polysyncraton sp.	XX	XXX	XXX	X	
Trididemnum orbiculatum	?	XXXX	XXXX	XXXX	
Trididemnum sp. 1	?	X	X	X	
Trididemnum sp. 2	?	?	?	X	
Ciona intestinalis	–	–	–	X	
Perophora multiclathrata	?	XX	XX	XX	
Perophora viridis	?	XX	XX	XX	
Perophora sp.	?	?	?	X	
Ascidia curvata	?	X	X	XX	Higher abundances on artificial substrate after 2000
Ascidia interrupta	?	X	X	XX	Higher abundances on artificial substrate after 2000
Ascidia cf multitentaculata	–	–	X	X	Only on artificial substrate
Ascidia sydneiensis	X	X	X	X	
Ascidia tenue	?	?	X	–	
Phallusia nigra	XXXX	XXXX	XXXX	XXXX	
Botrylloides giganteum	?	XXX	XXX	XXX	Only on artificial substrate
Botrylloides nigrum	XXXX	XXXX	XXXX	XXXX	
Botryllus tabori	XXX	XXX	XXX	XXX	
Botryllus tuberatus	?	XX	XX	X	
Cnemidocarpa irene	–	–	–	X	
Eusynstyela tincta	?	XX	XXX	XXX	
Eusynstyela sp.	–	–	X	XX	
Polyandrocarpa anguinea	X	X	X	X	
Polyandrocarpa zorritensis	X	–	X	XX	
Polycarpa spongiabilis	X	–	X	X	
Styela canopus	?	XXX	XXX	XXX	Only on artificial substrate
Styela plicata	X	X	X	X	Only on artificial substrate after 1980
Symplegma brakenhielmi	XXX	XXX	XXX	XXX	
Symplegma rubra	XXX	XXX	XXXX	XXXX	
Herdmania pallida	XXX	XXX	XXX	XXX	
Microcosmus exasperatus	?	XXX	XXX	XXX	
Pyura vittata	X	?	X	XX	
Molgula braziliensis	X	X	–	–	
Paraeugyrioides vannamei	X	?	?	X	

some *Diplosoma* with bilobed testicles and recognized *D. macdonaldi* as a distinct species, based on the absence of a muscular process. Kott (2001), however, agreed with Rowe (1966) and lists the Atlantic records as *D. listerianum*, without specific remarks. We recognize that the species has an unexpected distribution both in cold and tropical waters and might comprise a complex of cryptic species, but here we retain *D. listerianum*, while awaiting clarification of this matter.

https://doi.org/10.1017/S002531541200063X Published online by Cambridge University Press

Another taxonomic conundrum is *Distaplia* spp. from the Atlantic. Van Name (1945) recognized the synonymy proposed by Michaelsen (1930) between *D. bursata* (Van Name, 1921) and *D. stylifera* (Kowaleswsky, 1874) and considered the latter to be the valid name for the western Atlantic specimens. *Distaplia stylifera* has a broad range of variation in its characters and is probably a complex of cryptic species that needs urgent revision. The original record by Rodrigues *et al.* (1998) of a recently introduced species in SS used the name *Distaplia stylifera*, but we now recognize that this was a misidentification and further work may reveal that those animals are indeed a new species. Samples of a different *Distaplia* that more closely match the loose description of *D. stylifera* were recently (December 2009) collected at the Petrobras piers and at the Yacht Club of Ilha Bela on São Sebatião Island.

There are currently two species of *Symplegma* reported for the SSC: *Symplegma rubra* and *Symplegma brakenhielmi.* However, Couto (2003) reviewed the genus and recognized, but did not describe, two additional new species. Both *S. rubra* and *S. brakenhielmi* are widespread in warm waters and have very similar counterparts in the Indo-Pacific—the first with *S. bahraini* Monniot & Monniot, 1987 and the second with *Symplegma stuhlmanni* (Michaelsen, 1904). A revision of this genus including molecular analysis is necessary to understand the identity and geographical distribution of the species.

Eusynstyela sp. appeared for the first time during a monthly survey of rocky walls in Jarobá point in 1995. It was initially identified as *E. floridana* because of its small zooids, general colour and aspect of the colony. The study of the holotype (USNM 5969) revealed that both species differ in colony organization (Lotufo, 2002). *Eusynstyela floridana* forms clusters of zooids without apparent ordering, while in the SS the colonies are organized in a single layer of adjoined zooids, and the testis follicles are long and usually of the same size, while *E. floridana* has one dorsal globular follicle and one ventral and elongated follicle. *Eusynstyela* sp. resembles *E. hartmeyeri* Michaelsen, 1904, that is endemic to the Indian Ocean, and introduced in New Caledonia and Hong Kong (Monniot, 2002). In SSC, the colonies of *Eusynstyela* sp. are very conspicuous, in shallow waters and unlikely to remain undetected, and were not found in the region before 1995, suggesting that the species is also introduced in the SSC.

Despite the increase in species richness during the past 50 years due to the arrival of many alien species (ten reported during the past 15 years), important changes in species abundances were not noted during the same period. There are, however, exceptions, such as *Styela plicata*, *Polysyncraton* sp. and *D. rodriguesi* that decreased in abundance. Nonetheless, it is important to emphasize that abundance data are not precise enough to detect any but very large changes. *Styela plicata* is not native in the region and thus population fluctuations are expected. Causes of the decline in the two colonial species are unclear. However, both inhabit the intertidal zone, where conditions are naturally stressful for ascidians and where the first effects of environmental degradation are frequently observed during oil-leaks or by sewage release (Peterson *et al.*, 2003).

The SSC is sheltered, protected by São Sebastião Island, has many marinas and is one of the most important oil terminals in Brazil. By the end of 2000, 220 oil leaks were reported for the SSC, and during a single event in May 1994, 2.6 million l of oil leaked from the oil terminal into the SSC. These constant accidents, along with untreated domestic pollution that enters the waters, increase the organic load and thereby affect the local fauna (Cunha, 2003). Reports from the state environmental agency, CETESB, from 1991 to 2008 showed a trend of an increasing number of beaches with coliform indices above the legal limits. For instance, in 1999, 65% of the beaches were considered adequate in the SSC, while in 2008 that had declined to only 40% of the beaches (CETESB, 2001, 2009). Impacts on the biota have been shown in molluscs, where the number of faecal coliform bacteria increases during the summer (vacation period) and this increase is associated with reduced mollusc species richness and diversity (Denadai *et al.*, 2001). While we have no data for ascidians, we infer that these sessile animals, with their filter feeding habits, are affected by eutrophication. In Jamaican lagoons, eutrophication was the main reason for the changes in the ascidian fauna during the course of 30 years (Goodbody, 1993). On the other hand, bacterial increase was mentioned as the main cause of a population explosion of *Trididemnum solidum* in Curaçao that culminated in reduction of local species richness (Bak *et al.*, 1996).

Among the introduced species present in the SS region, some were reported elsewhere as invasive, for instance, *Ciona intestinalis* (Braithwaite & McEvoy, 2005; Howes *et al.*, 2007), *Styela plicata* (Rocha *et al.*, 2009) and *Didemnum perlucidum* (Lambert, 2002). *Ciona intestinalis* and *S. clava* are causing considerable damage to mussel farms in Canada because of high costs associated with the cleaning of shells and submerged structures (Carver *et al.*, 2003; Davis & Davis, 2009). Rodrigues *et al.* (1998) included *C. intestinalis* in the ascidian guide for the State of São Paulo, but only one animal had been collected at that time. Vieira *et al.* (2012) during an experiment conducted in 2007 at Segredo Beach found several specimens of *C. intestinalis* on PVC plates protected against predation. Inspections along the beach did not find evidence that the species occurred on natural substrates. Similar effects of predation on *C. intestinalis* were reported in Guanabara Bay, Brazil (Marins *et al.*, 2009) and Chile (Dumont *et al.*, 2011). However, one individual was found on a natural substrate on a more distant island near SS (Rocha & Bonnet, 2009). *Styela plicata* was very common on natural substrates in shallow waters in the 1960s (Rodrigues, 1962) but it became uncommon and restricted to artificial, usually hanging, substrates. Both species usually live in waters colder than the usual water temperatures in SS (Yamaguchi, 1975) and peaks of high water temperature may control their populations. For instance, high mortality of *S. plicata* occurred in oyster farms in Santa Catarina, south of Brazil, in March 2008 when surface water temperatures reached 29–30°C for several consecutive days (Nelson Silveira Jr, personal communication). *Didemnum perlucidum* is abundant on artificial substrates in the SSC with very small colonies on the underside of boulders, suggesting that the species might have been controlled by predation in natural habitats. Given that *D. perlucidum* has a great capability to colonize new substrates (Kremer *et al.*, 2010) and that other didemnid species are known for their rapid growth rates and space domination, causing a decrease in local species richness (*Didemnum vexillum*—Bullard *et al.*, 2007; *Trididemnum solidum*—Bak *et al.*, 1996; *Diplosoma similis*—Vargas-Ángel *et al.*, 2009), *D. perlucidum* and other introduced didemnids in the region may be a threat for natural communities and should be monitored for any signs of population increase.

https://doi.org/10.1017/S002531541200063X Published online by Cambridge University Press

Speculation about temporal variation in populations of species occurring in deep waters is not possible because most of the sampling efforts during the past 50 years were concentrated on rocky shores from the intertidal to 10 m depth and hence many species that occur outside that zone were rarely observed. *Euherdmania vitrea* is one of these species that occurs in areas deeper than 10 m and so was only occasionally found, mainly by dredging studies. Yet, the species is common in shallow (6–8 m) waters of Santa Catarina, southern Brazil (Rocha *et al.*, 2005b), which suggests that the SS population could be a marginal, northern-most geographical limit for this species. In addition, species living in soft bottoms, such as *Paraeugyrioides vannamei*, have certainly been underestimated by our studies.

Any monitoring in the region must also take into account that the abundance of many species, such as *Clavelina oblonga* and *Diplosoma listerianum*, oscillates throughout the year and sometimes a marked seasonal decline occurs in winter. *Clavelina oblonga* can dominate the encrusting community at the end of summer and beginning of autumn (March–April) on both artificial and natural substrates. *Diplosoma listerianum* has a fragile tunic, rapid colony growth and early maturation of gonads (Rocha, 1991). During the spring and summer *D. listerianum* can become very common and form large colonies, but usually has high mortality soon after larval recruitment and colony growth, disappearing in subsequent months when small colonies hidden in protected places are occasionally found.

In conclusion, while the SS region has one of the best-known ascidian faunas in Brazil, it is also one of the most challenging in terms of marine conservation. Difficulties arise from its location in the most urbanized and industrialized state in Brazil, and because the SSC is considered a key piece in the economic development of the region. Urban and industrial pollution, biological invasions and construction of coastal facilities and structures may be considered the main threats to marine organisms in general, and to ascidians in particular. This study provides a current detailed description of the ascidian species in the area and a baseline for future taxonomic studies, conservation of native species and management of introduced species. To the best of our knowledge, the data reported here comprise the first account of a long-term ascidian survey in the south-western Atlantic, an area which is still relatively poorly known.

ACKNOWLEDGEMENTS

The authors thank Dr Sérgio de Almeida Rodrigues (in memoriam) who introduced R.M.R., T.M.C.L. and G.M.D. to the study of ascidians, the Center of Marine Biology (CEBIMar-USP) and Yatch Club Ilhabela for field support and James J. Roper for his review of the English. We also thank CNPq for a research grant (R.M.R.) and financial support, FAPESP for providing grants for G.M.D. and T.M.C.L. and for financing field trips in the region coordinated by Dr Roberto G.S. Berlinck and CAPES who provided graduation grants for G.M.D. and who financed the December 2009 RAS in the region.

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