

**Research article****Human-mediated global dispersion of *Styela plicata* (Tunicata, Ascidiacea)**

Rodolfo C. de Barros, Rosana M. da Rocha\* and Marcio R. Pie

Departamento de Zoologia, Universidade Federal do Paraná, C.P. 19020, 81.531-980, Curitiba, PR, Brazil

\*Corresponding author

E-mail: [rmrocha@ufpr.br](mailto:rmrocha@ufpr.br)

Received 8 February 2008; accepted for special issue 19 April 2008; accepted in revised form 29 December 2008; published online 16 January 2009

**Abstract**

*Styela plicata* (Lesueur, 1823) is a solitary ascidian found in shallow, protected environments in tropical and warm-temperate oceans. Its origin is uncertain, given that it has already been identified in several oceans since it was first described, showing a very broad geographical distribution. Although *S. plicata* has been historically classified as a cosmopolitan species, in the past few decades it has been considered as an introduced or invasive species in some regions of the world. The present study investigated the genetic variation among populations of *S. plicata*. A total of 51 samples were obtained from locations on the Atlantic and Pacific coasts of the USA, Japan, and southern and southeastern Brazil. The amplification of a fragment of the cytochrome oxidase subunit I gene (COI) generated nine distinct haplotypes. There was considerable variation in the geographical distribution of haplotypes, yet the highest nucleotide and haplotypic diversities were clearly found in the Pacific samples. Interestingly, one of the haplotypes showed more than 3% divergence in relation to the remaining haplotypes, suggesting the possibility of a cryptic species. These results, together with historical records, indicate that commercial shipping could be the main cause for the global distribution of *S. plicata*. The northwestern Pacific region is hypothesized as the center of distribution of the species.

**Key words:** bioinvasion, COI, dispersal routes, NIS, sea squirt, Urochordata**Introduction**

Ascidiaceans are considered as an excellent model system for biogeographical studies (Monniot 1983), given that the adult stage is sessile and natural dispersal occurs through gametes or larvae (Lambert 2005). Under natural conditions, colonial ascidian larvae usually do not disperse more than a few meters. Larvae of solitary species, on the other hand, can remain swimming freely for periods longer than 12 h, causing a consequent broader distribution (Ayre et al. 1997).

*Styela plicata* (Lesueur, 1823) is a solitary ascidian found in shallow, protected environments in tropical and warm-temperate oceans (Figure 1). Its geographical distribution is broad, yet its origin is unknown (Lambert 2001). The type-specimen was found attached to the hull of a ship in Philadelphia, although no other individual was detected in natural substrates in the region (Van Name 1945). Other records of the presence of *S. plicata* on ship hulls have been

made in the Bay of Hann, Senegal, in 1950 (Pérès 1951) and on the USS Palos after a voyage through the Pacific, coming from either China or Japan (Tokioka 1967).

The species has been recorded in warm, temperate waters of the Atlantic Ocean and the Mediterranean (Annex 1, Harant 1927; Harant and Vernières 1933). Although it has been found throughout much of the eastern coast of North America since the beginning of the 20<sup>th</sup> century (Van Name 1912; Huntsman 1912), it had been considered very rare on the west coast until the mid 1940's (Van Name 1945). It had not been recorded in Bermuda at the beginning of the 20<sup>th</sup> century (Van Name 1902), until it was first reported as a single specimen in 1945 (Van Name 1945) and as a small population in 1972 (Monniot 1972). It was considered as an introduced species in the Gulf of Mexico (Lambert et al. 2005). The earliest record in the Pacific Ocean is in the Sydney harbor, Australia, in 1878 (Heller 1878), but it is also considered as an introduced species in that region (Kott 1985;

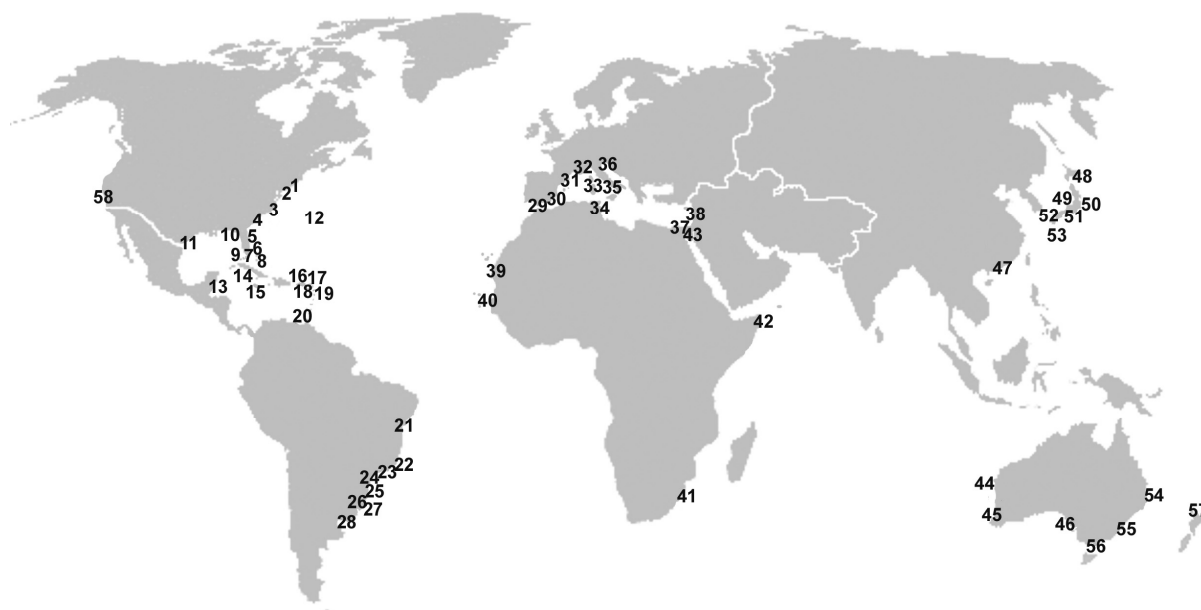


Figure 1. World distribution of *Styela plicata*. Location details in Annex 1.

Berents and Hutchings 2002; Wyatt et al. 2005), whereas it was first reported in New Zealand in 1948 (Brewin 1948). There were no occurrence records on islands and reefs in tropical waters in either the east or west Pacific until 1980 (Kott and Goodbody 1980). It is invasive in southern Brazil (Rocha and Kremer 2005) and southern California (Lambert and Lambert 2003).

*Styela plicata* thrives on brackish and polluted waters, frequently being found in estuarine environments (Kott 1952, 1972a; Kott and Goodbody 1980) or in shallow waters surrounding the mainland where large flows of freshwater reduce local salinity (Sims 1984). It is also found in disturbed areas such as in the proximity of refineries, power plants, and fishing harbors (Carballo and Naranjo 2002). It can be considered as an indicator species in areas that have experienced intense stress (substrate transformation, water stagnation, and excessive sedimentation) for extended periods of time (Naranjo et al. 1996). It can adhere to several types of substrate, particularly artificial substrates, and is also found in epibiosis. *Styela plicata* occurs on the shells of bivalves and competes with them for resources (Perera et al. 1990), and can also prey on their larvae (Bingham and Walters 1989). These features cause the presence of *S. plicata* to be highly undesirable in shellfish aquaculture.

The goal of the present study is to investigate the evolutionary relationships among seven populations of *S. plicata*, including samples from both the Atlantic and Pacific oceans, through the study of sequence variability in a fragment of the cytochrome oxidase subunit I gene. The comparison of samples of varying geographical distances is used to understand the dispersion patterns of the species, as well as to infer its geographical origin.

## Methods

### Specimen collection

Seven populations of *S. plicata* were sampled across four geographically distinct regions. Two populations were in the Pacific Ocean and the remaining populations were in either North or South Atlantic Oceans (Table 1). Samples of two species were used as outgroups in the analyses: *Polycarpa pomaria* (Savigny, 1816) (obtained from GenBank under accession number AY600984.1) and *Styela canopus* (Savigny, 1816) (collected on August 11, 2006 in Bocas del Toro, Panama).

Specimens were dissected in the field or in the laboratory for the removal of tissue fragments (muscles and gonads), which were stored in 95% ethanol and kept at approximately -18°C (Hillis et al. 1996).

**Table 1.** Studied populations of *Styela plicata*.

Country	Site	Coordinates	Date of collection
Japan	Sakushima Island	34°43'N, 137°02'E	7 August 2006
USA	Santa Barbara, California	34°25'N, 119°41'W	16 December 2005
	Cape Canaveral, Florida	28°27'N, 80°32'W	22 and 24 August 2005
Brazil	Enseada de Jurujuba, Rio de Janeiro	22°56'S, 43°07'W	17 February 2006
	Ilha do Mel, Paraná	25°30'S, 48°23'W	14 November 2005, 8 January 2006
	Penha, Santa Catarina	26°47'S, 48°39'W	12 December 2005
	Ribeirão da Ilha, Santa Catarina	27°43'S, 48°34'W	29 April 2006

### DNA extraction and sequencing

DNA was extracted using a modification of the EZ-DNA protocol (Biosystems®), with digestion buffer (Tris 50mM pH 8.0) and proteinase K (Biosolv Lid), at a concentration of 25 mg/mL. DNA concentrations were measured following extraction using a Genequant *pro* spectrophotometer (Amersham Biosciences).

Universal primers HCO1498 (5'- TAA ACT TCA GGG TGA CCA AAA ATC - A 3') and LCO2198 (5'- GGT CAA CAA ATC ATA AAG ATA TTG G - 3') were used to amplify approximately 700 pb of the cytochrome oxidase subunit I gene, according to Folmer et al. (1994). PCR amplification was carried out in 25- $\mu$ L solutions using the following final concentrations: 1 X reaction buffer, 3 mM of MgCl<sub>2</sub>, 0.4 mM of dNTP (Biotools BeM Labs), 50  $\mu$ mol of each primer, 1 U of Taq Platinum DNA polymerase (Invitrogen) and 10-50 ng of template DNA. Thermocycling conditions had the following specifications: 2 min at 95°C, followed by 35 cycles of 40 s at 92°C, 40 s at 57°C, and 40 s at 70°C, and a final extension for 5 min at 70°C. After purification with ammonium acetate (7.5 M), sequencing reactions were carried out in a 10- $\mu$ L volume containing 0.5  $\mu$ L of BigDye (Terminator v3.1 Cycle Sequencing Kit - Applied Biosystems), 1.0  $\mu$ L of primer (1.6  $\mu$ mol), 0.5  $\mu$ L of buffer solution, 0.5  $\mu$ L to 6.0  $\mu$ L of DNA and ultrapure water, with the following thermocycling conditions: 1 min at 96°C, followed by 35 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C. Sequencing was carried out on a 3130 Applied Biosystems automatic sequencer.

### Data analysis

Electropherograms were reconciled using the software BioEdit v.5.0 (Hall 1999) and aligned unambiguously due to the absence of indels.

A phylogenetic tree of the studied haplotypes was inferred using the neighbor-joining (NJ) method (Saitou and Nei 1987), with topology and bootstrap support values (1000 replicates) being calculated using the software PAUP\* 4.0 (Swofford 2000) based on a GTR+ $\Gamma$  model of sequence evolution. This model was selected by comparing the likelihood values of alternative models using the Akaike information criterion, as implemented in Modeltest (Posada and Crandall 1998). These results were complemented by an additional analysis using Bayesian Inference (BI) using to the GTR+ $\Gamma$ +I model (2.500.000 generations, with a burn-in of 1000 generations), as implemented in the software MrBayes 3.0 (Ronquist and Huelsenbeck 2003).

Diversities at both nucleotide ( $\pi$ ) and haplotype (Hd) levels (Nei 1987) were measured in each population using the software DnaSP v.4.1 (Rozas et al. 2003). The hypothesis of a nonrandom distribution of haplotypes (as opposed to panmixia) among populations of different locations was assessed using the exact test for population differentiation (Raymond and Rousset 1995), with its significance being measured from 10.000 random permutations in the software Arlequin 3.1 (Excoffier et al. 2005).

### Results

A total of 51 sequences (562 bp) of the studied COI fragment were obtained for *S. plicata* and were classified into nine distinct haplotypes (Table 2, GenBank accession numbers EU708881-EU708889). Twenty-three polymorphic sites were identified (4.1%), four of which represented nonsynonymous substitutions (Figure 2). Transitions accounted for approximately 77% of the substitutions, of which 77.3% were located on the third codon position and 22.7% on the second position. The mean AT

content of the sequences was 63.2%. Nucleotide diversity ( $\pi$ ) and haplotype diversity for the entire dataset were  $0.0095 \pm 0.002$  and  $0.784 \pm 0.05$ , respectively (mean  $\pm$  SD).

The phylogenetic relationships among the haplotypes of *S. plicata* are shown in Figure 3. The results from BI and NJ analyses were highly concordant, except for the expected instability in the very short nodes (indicated in Figure 3 as the nodes without support values). There was considerable geographical variation in the distribution of haplotypes, with the same haplotype being found in very distant locations, as one would expect in a highly invasive species. Interestingly, haplotype 9 is highly divergent in relation to all other haplotypes and might in our opinion represent a distinct species. Therefore, henceforth the text will focus only on the remaining 8 haplotypes.

The studied haplotypes could be divided into two groups. The first group includes haplotypes 1-4 and is most commonly found in the West Atlantic, except for two occurrences of haplotype 2 in Japan. The second group includes haplotypes 5-8 and is monophyletic using the NJ method (but with only 33% bootstrap support) but not under the BI method (Figure 3), possibly due to the fairly high divergence between haplotype 9 and the remaining sequences. The haplotypes in the second group tend to be more geographically widespread, such as haplotypes 7 and 8 (Table 2). Such differences are also reflected in a statistically significant heterogeneity in the distribution of haplotypes ( $P < 0.05$ ) based on an exact test for population differentiation (Raymond and Rousset 1995). Interestingly, although the dataset included more samples from the Atlantic, the highest haplotype

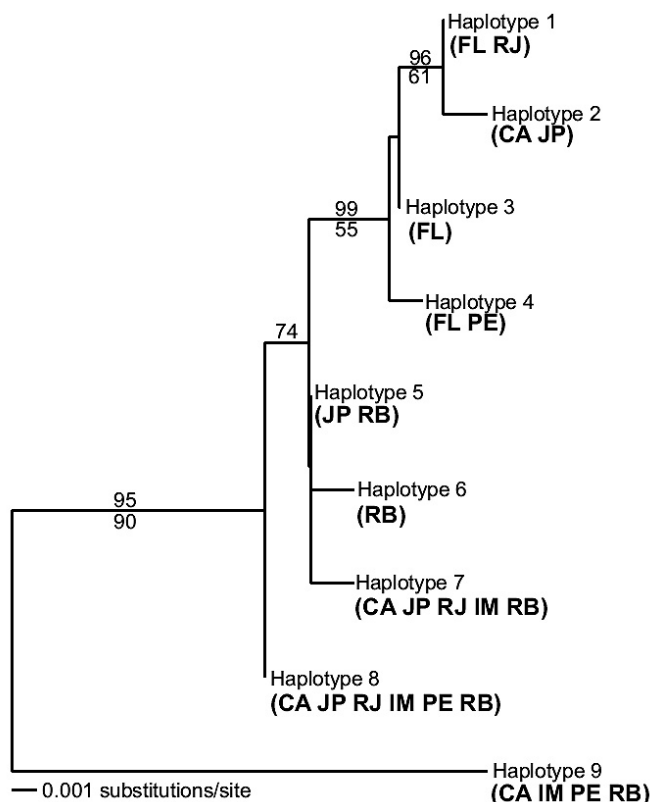
**Table 2.** *Styela plicata*. Haplotype frequencies, nucleotide diversity ( $\pi$ ) and haplotype diversity (Hd) within populations, and number of polymorphic sites in the studied populations. The haplotype 9 was not considered in obtaining  $\pi$ , Hd and polymorphic sites.

<i>Styela plicata</i> populations	Haplotypes									N	$\pi$	Hd	Polymorphic sites
	1	2	3	4	5	6	7	8	9				
Florianópolis (RB)				1	1	1	1	4	2	9	0.00203	0.714	3
Penha (PE)				1				4	1	6	0.00285	0.400	4
Ilha do Mel (IM)							2	4	1	7	0.00190	0.533	2
Rio de Janeiro (RJ)	1						1	4		6	0.00332	0.600	5
Florida (FL)	1		3	3						7	0.00153	0.714	2
California (CA)		1					2	2	2	7	0.00498	0.800	6
Japan (JP)		2			1		3	3		9	0.00455	0.806	6
Totals	2	3	3	4	2	1	9	21	6	51	0.00387	0.737	8

				*	*		*								*									
		2	80	122	129	131	140	161	227	258	266	267	278	296	299	305	311	335	344	407	440	501	515	549
Hap 1	(2)	T	T	C	C	T	T	C	C	A	T	A	T	C	A	T	T	A	C	G	A	C	G	C
Hap 2	(3)	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
Hap 3	(3)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.
Hap 4	(4)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Hap 5	(2)	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A
Hap 6	(1)	.	.	.	A	.	.	.	.	.	C	.	.	.	.	.	C	.	.	.	.	.	.	A
Hap 7	(9)	C	.	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	A
Hap 8	(21)	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	T	.	A
Hap 9	(6)	.	G	T	A	C	C	T	T	G	.	.	C	T	T	C	C	G	T	T	G	T	A	A

**Figure 2.** Sequence variation among the studied haplotypes of *Styela plicata*. Above numbers represent the position of the variable sequences, and asterisks indicate nonsynonymous substitutions. The number of individuals of each haplotype are shown in parenthesis. See text for details.

**Figure 3.** Phylogenetic relationships among haplotypes of *Styela plicata* based on the Neighbor-Joining method. The number above a node indicates its posterior probability (according to the Bayesian Inference method), whereas the number below the node indicates NJ bootstrap support. Only support values above 50% are shown. The outgroup taxa are not shown to facilitate the visualization of the relationships among haplotypes. Codes below each haplotype correspond to the location where it was found: RB - Florianópolis, PE - Penha, IM - Ilha do Mel, RJ - Rio de Janeiro, FL - Florida, CA – California, and JP - Japan.



frequencies were observed in the Pacific, particularly in California (Hd=0.800) and Japan (Hd=0.806). The same pattern was observed for nucleotide diversities, which were nearly twice that in the Atlantic. These results are consistent with the Pacific being the source for the dispersal of *S. plicata*. Moreover, the occurrence of South American samples in both groups of haplotypes (Figure 3) might indicate two separate invasions of *S. plicata* into the Atlantic coast of South America.

### Discussion

The results clearly indicate considerable geographical variation in the distribution of haplotypes of *Styela plicata*. Two scenarios could be invoked to explain this pattern: either the species is very ancient, with a widespread distribution followed by several local extinction events, or the species has experienced significant transport through human vessels, such that the observed pattern would be the result from multiple local introductions. Two lines of evidence support the second scenario. The species was recorded for

the first time in the Pacific, Atlantic and Mediterranean only in the 19<sup>th</sup> century. Moreover, an approximate date of introduction can be inferred in several locations, such as California (Ritter and Forsyth 1917), Bermuda (Van Name 1945), regions to the west of the Sydney Harbor (Kott 1972a), and New Zealand (Brewin 1948), based on the fact that previous surveys had failed to indicate the presence of the species. *Styela plicata* is a large species with straightforward identification characters, suggesting that its presence would likely not remain unnoticed. In addition, most records have been made in regions near harbors, particularly on artificial substrates (Annex 1), which also supports its status as an invasive species.

*Styela plicata* has several biological characteristics that might have contributed to its dispersion capacity. Under experimental conditions (26°C and salinity 34‰), the larva takes on average 4.5 h to attach to a substrate after the hatching from its egg. However, they can continue to swim for up to two days without compromising their metamorphosis or post-larval development (Thiyagarajan and Qian 2003).

Even damaged embryos can still develop into larvae and potentially generate functional adults (Nakauchi and Takeshita 1983). Within only four days after settlement, the functional ascidian is completely formed in temperatures around 24–25°C. The animals are sexually mature at 40 mm, and live for five to nine months (Yamaguchi 1975). The species does not seem to be sexually active during the colder months, but can still attain two to three reproductive generations per year in Japan (Yamaguchi 1975). The rapid growth and the high reproductive rates allow the species to rapidly and intensively colonize new substrates, leading to large populations (Morris et al. 1980). The species can be highly resistant to anti-fouling paints (Raftos and Hutchinson 1997). Finally, the presence of secondary metabolites on the body wall of *S. plicata* causes it to be unpalatable to predators, particularly fish (Pisut and Pawlik 2002).

Although the dispersion of many ascidian species is a natural phenomenon on smaller scales, particularly through water currents or attached to floating objects (Carlton 1987), anthropogenic transportation has been the main cause of successful invasions of exotic ascidian species (Lambert 2005). The presence of ascidians in harbors and the movement of vessels contribute considerably to their transport between continents (Monniot et al. 1985), with adults attached to vessel hulls being the most likely vectors (Carlton and Geller 1993; Lambert 2001). More recently, the transport inside sea-chests has become increasingly important, given that the water friction during travel is minimal (Coutts and Dodgshun 2007). The historical records demonstrating the occurrence of *S. plicata* in several harbor regions and the presence of individuals of the species traveling attached to ship hulls strongly support a link between its dispersal and global navigation. International maritime transport is associated with nearly 80% of the world's commerce (Carlton 2001). In southern Brazil, more than 22,000 ships went through the Paranaguá and Antonina harbors between 1995 and 2006, with a nearly 70% increase in activity in this period (<http://www.portosdoparana.com.br>). The traffic in the Itajaí harbor more than doubled between 1999 and 2006, with nearly 6,400 moored ships. This harbor has weekly maritime connections with northern Europe, the Mediterranean, the Gulf of Mexico (USA and Mexico), the western Atlantic (USA and Canada), the eastern Pacific (EUA), the Caribbean (Jamaica, Puerto Rico,

Bahamas, Dominican Republic), and Japan (<http://www.portoitajai.com.br>). These data reflect the growth tendency for maritime commerce and the increased risk of new introductions.

The genetic structure of an invasive species depends on several factors, including the effective size of the introduced population and the genetic diversity of the source population. In general, invasive populations have low genetic variability, particularly if the introduction takes place as a single event and involves few founders (Holland 2000). This seems to be the case in the invasive ascidian *Botryllus schlosseri* Pallas, 1766, which was introduced to the harbors of southern Europe (López-Legentil et al. 2006). The fairly high haplotypic diversity observed in the present study (average  $Hd=0.737\pm 0.03$ , without haplotype 9) seems therefore to contradict that prediction. However, recent studies have demonstrated that it is possible to find high genetic variability in invasive species, both sessile (Turon et al. 2003) and vagile (Barbaresi et al. 2003). For instance, the crab *Carcinus maenas* Linnaeus, 1758 showed similar levels of haplotypic diversity in its native (Roman and Palumbi 2004) and introduced regions (Roman 2006). Therefore, the expected effects of genetic drift following the introduction can be mitigated if the founder individuals are sufficiently diverse genetically, or if the process involves several independent rounds of invasion. In fact, the historical record of the colonization of *S. plicata* in different regions (1823 and 1843, in the north Atlantic; 1883 in the south Atlantic and Mediterranean; 1894 in the North Pacific, and 1877 in the South Pacific) and the distribution of haplotypes observed in this study suggest the possibility of complex rounds of invasion through different routes.

The exceptionally high levels of nucleotide and haplotypic diversity in the Pacific samples (Table 2) suggests that this region represents the center of dispersion of *S. plicata*. In particular, the sample from Japan showed not only the highest genetic variability levels, but also includes the most ancient haplotype (haplotype 8, Figure 3). Indeed, it is a common pattern inferred from the neutral theory that the most common haplotype is probably the most ancient (Watterson and Guess 1977). A connection between Asia and California had been suggested before (Ruiz et al. 1997), and the very high genetic variability in California might be due to repeated invasion from the Northeastern Pacific.

A surprising result from the phylogenetic analysis of *S. plicata* haplotypes is the possibility that haplotype 9 might in fact represent a distinct species. Several lines of evidence corroborate this claim. First, it has consistently been identified as the most divergent haplotype (nearly 3% divergent from the remaining haplotypes). Second, it has been consistently placed outside the clade including all the remaining haplotypes with very strong support from all analyses. Finally, it has been found sympatrically with other haplotypes in several locations, indicating that such high level of divergence is not due to geographical differentiation. Further studies, particularly including nuclear markers, will be instrumental to corroborate this hypothesis and to elucidate the relationship of haplotype 9 to *S. plicata*. If the existence of this new cryptic species is indeed confirmed, all the previous records of *S. plicata* should be reviewed, but we suspect that most of them are correctly assigned to *S. plicata*, given that most of the haplotypes are evidently from this species.

Several studies have demonstrated the effectiveness of mtDNA in bioinvasion studies to elucidate dispersion routes and centers of origin (Geller et al. 1997; Turon et al. 2003; Mackie et al. 2006). However, analyses based on a single molecular marker should be regarded with caution. The present study indicates a complex pattern of dispersal of haplotypes of *S. plicata*. Molecular and historical evidence suggest that the broad geographical distribution of *S. plicata* has an anthropogenic origin, with northwestern Pacific being the possible center of origin of the species. Future studies, including more extensive sampling of the northeastern Pacific (Japan, Korea, China, and Russia) and additional loci will allow for a better description of the origin and dispersion of *S. plicata* from that region.

### Acknowledgements

We thank Flávia de Oliveira Marins (Universidade Federal do Rio de Janeiro), Shota Chiba (University of California), Kristen Larson (Smithsonian Environmental Research Center, Florida) and Teruaki Nishikawa (Nagoya University, Japan) for kindly providing specimens. Dr. Walter A. P. Boeger also supplied lab facilities for DNA sequencing analyses. CNPq gave a research grant to RMR. This is contribution 1736 of Zoology Department, Universidade Federal do Paraná.

### References

- Abbott DP, Johnson JV (1972) The ascidians *Styela barnharti*, *S. plicata*, and *S. montereyensis* in Californian waters. *Bulletin of the Southern California Academy of Sciences* 71: 95-105
- Ayre DJ, Davis AR, Billingham M, Llorens, Styan C (1997) Genetic evidence for contrasting patterns of dispersal in solitary and colonial ascidians. *Marine Biology* 130: 51-61, doi:10.1007/s002270050224
- Barbaredi S, Fani R, Gherardi F, Mengoni A, Souty-Grosset C (2003) Genetic variability in European populations of an invasive American crayfish: preliminary results. *Biological Invasions* 5: 269-274, doi:10.1023/A:1026133519707
- Berents P, Hutchings P (2002) Port survey for introduced marine species - Sydney Harbour - Final report. Australian Museum Business Services, Sydney
- Bingham BL, Walters LJ (1989) Solitary ascidians as predators of invertebrate larvae: evidence from gut analyses and plankton samples. *Journal of Experimental Marine Biology and Ecology* 131: 147-159, doi:10.1016/0022-0981(89)90004-X
- Brewin BI (1948) Ascidians of the Hauraki Gulf. Part I. *Transactions of the Royal Society of New Zealand* 77: 115-138
- Brewin BI (1958) Ascidians of the Hauraki Gulf. Part III. *Transactions of the Royal Society of New Zealand* 85: 455-458
- Brunetti R (1979) Ascidians of the Venice Lagoon I. Annotated inventory of species. *Annales de l'Institut Océanographique* 55(1): 95-109
- Carlton JT (1987) Patterns of transoceanic marine biological invasions in the Pacific Ocean. *Bulletin of Marine Science* 41(2): 452-465
- Carlton JT (2001) Introduced species in U.S. coastal waters: Environmental impacts and management priorities. Pew Oceans Commission, Virginia
- Carlton JT, Geller JB (1993) Ecological roulette: The global transport of nonindigenous marine organisms. *Science* 261: 78-82, doi:10.1126/science.261.5117.78
- Carballo JL, Naranjo S (2002) Environmental assessment of a large industrial marine complex based on a community of benthic filter-feeders. *Marine Pollution Bulletin* 44: 605-610, doi:10.1016/S0025-326X(01)00295-8
- Costa HR (1969) Notas sobre os Ascidiacea brasileiros. VI. *Atas Sociedade de Biologia do Rio de Janeiro* 12(5-6): 321-325
- Coutts ADM, Dodgshun T (2007) The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. *Marine Pollution Bulletin* 54: 875-886, doi:10.1016/j.marpolbul.2007.03.011
- De Kay JE (1843) Natural history of New York. Albany: New York Assembly.
- Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47-50
- Fay RC, Johnson JV (1971) Observations on the distribution and ecology of the littoral ascidians of the Mainland coast of southern California. *Bulletin of the Southern California Academy of Sciences* 70(3): 114-124
- Fiala-Medioni A (1974) Ascidiés du benthos rocheux de Banyuls-Sur-Mer. Inventaire faunistique et notes écologiques. *Vie Milieu* 24(1): 193-207

- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299
- Gabriele M, Putrone V, Brunetti R (1997) Morphometrics and energetic value of Adriatic ascidians. *Cahiers de Biologie Marine* 38: 169-174
- Geller JB, Walton ED, Grosholz ED, Ruiz GM (1997) Cryptic invasions of the crab *Carcinus* detected by molecular phylogeography. *Molecular Ecology* 6: 901-906, doi:10.1046/j.1365-294X.1997.00256.x
- Ghobashy AFA, Abdel Messeih MK (1991) Ascidians in Egyptian waters. *Journal of the Egyptian-German Society of Zoology* 4: 313-326
- Goodbody I (1984) Ascidians from Caribbean shallow water localities. *Studies on the fauna of Curaçao and other Caribbean islands* 203: 62-76
- Goodbody I (2003) The Ascidian fauna of Port Royal, Jamaica. I. Harbor and mangrove dwelling species. *Bulletin of Marine Science* 73(2): 457-476
- Goodbody I (2004) Diversity and distribution of Ascidians (Tunicata) at Twin Cays, Belize. *Atoll Research Bulletin* 524: 1-19
- Harant H (1927) La faune ascidiologique de Banyuls et de Cette: essai de révision des ascidies de la Méditerranée occidentale. *Annales de l'Institut Océanographique de Monaco* 4: 209-251
- Harant H, Vernières P (1933) Faune de France - Tuniciers - Fasc. 1: Ascidies 27:1-101
- Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 40: 413-427
- Heller C (1878) Beitrage zur nahern Kenntnis der Tunicaten. *Sitzber Akad Wiss Wien* 77(1): 2-28
- Hillis DM, Moritz C, Mable BK (eds) (1996) Molecular systematics. Sinauer, Massachusetts
- Holland BS (2000) Genetics of marine bioinvasions. *Hydrobiology* 420: 63-71, doi:10.1023/A:1003929519809
- Huntsman AG (1912) Ascidians from the coasts of Canada. *Transactions of the Royal Society of Canada. Section I* 9: 111-148
- Kott P (1952) The Ascidians of Australia. I - Stolidobranchiata Lahille and Phlebobranchiata Lahille. *Australian Journal of Marine and Freshwater Research* 3(3): 205-233, doi:10.1071/MF9520205
- Kott P (1972a) Some sublittoral ascidians in Moreton Bay, and their seasonal occurrence. *Memoirs of the Queensland Museum* 16(2): 233-260
- Kott P (1972b) The Ascidians of South Australia II. Eastern sector of the Great Australian Bight and Investigator Strait. *Transactions of the Royal Society of South Australia* 96(4): 165-196
- Kott P (1973) Notes on some Ascidians from Port Jackson, Botany Bay and Port Hacking, New South Wales. *Proceedings of the Linnean Society of New South Wales* 97(4): 241-257
- Kott P (1985) The Australian Ascidiacea - Phlebobranchia and Stolidobranchia. *Memoirs of the Queensland Museum* 23: 1-440
- Kott P, Goodbody I (1980) The ascidians of Hong Kong. In: Morton BS, Tseng CK (eds) Proceedings of the first international marine biological workshop: the flora and fauna of Hong Kong and Southern China. Hong Kong University Press, Hong Kong
- Lambert CC, Lambert G (2003) Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. *Marine Ecology Progress Series* 259: 145-161, doi:10.3354/meps259145
- Lambert G (2001) A global overview of ascidian introduction and their possible impact on the endemic fauna. In: Sawada H, Yokosawa H and Lambert CC (eds) The Biology of Ascidians. Springer-Verlag, Tokyo
- Lambert G (2005) Ecology and natural history of the protochordates. *Canadian Journal of Zoology* 83:34-50, doi:10.1139/z04-156
- Lambert G, Faulkes Z, Lambert CC, Scofield VL (2005) Ascidians of South Padre Island, Texas, with key to species. *Texas Journal of Science* 57: 251-262
- Lesueur CA (1823) *Ascidia plicata*. Descriptions of several new species of *Ascidia*. *Journal of the Academy of Natural Sciences of Philadelphia* 3(1): 2-8
- Leung Tack Kit D (1975) Étude qualitative et quantitative des salissures biologiques de plaques expérimentales immergées en pleine eau. 5 - Les ascidies. *Tethys* 7(2-3):223-234
- López-Legentil S, Turon X, Planes S (2006) Genetic structure of the star sea squirt, *Botryllus schlosseri* introduced in southern European harbours. *Molecular Ecology* 15: 3957-3967, doi:10.1111/j.1365-294X.2006.03087.x
- Lotufo TMC (2002) Ascidiacea (Chordata: Tunicata) do litoral tropical brasileiro. Dissertation, Universidade de São Paulo
- Mackie JA, Keough MJ, Christidis L (2006) Invasion patterns inferred from cytochrome oxidase I sequences in three bryozoans, *Bugula neritica*, *Watersipora subtorquata* and *Watersipora arcuata*. *Marine Biology* 149: 285-295, doi:10.1007/s00227-005-0196-x
- Millar RH (1955) On a collection of ascidians from South Africa. *Proceedings of the Zoological Society of London* 125 (1): 169-221
- Millar RH (1958) Some ascidians from Brazil. *Annals and Magazine of Natural History* 13(1): 497-514
- Millar RH (1966) Ascidiacea - Port Phillip Survey 1957-1963. *Memoirs of the National Museum - Victoria* 27: 357-384
- Millar RH (1982) The marine fauna of New Zealand: Ascidiacea. *New Zealand Oceanographic Institute Memoir* 85: 1-117
- Millar RH (1988) Ascidians collected during the International Indian Ocean Expedition. *Journal of Natural History* 22: 823-848, doi:10.1080/00222938800770541
- Monniot C (1969) Sur une collection d'ascidies de Dakar (Phlébobranches et Stolidobranches). *Bulletin du Muséum National d'Histoire Naturelle* 41(3): 622-654
- Monniot C (1969-1970) Ascidies Phlébobranches et Stolidobranches. *Resumes Scientifiques de la Campagne du Calypso* 9: 03-59
- Monniot C (1972) Ascidies Stolidobranches des Bermudes. *Bulletin du Muséum National d'Histoire Naturelle* 57: 617-643
- Monniot C (1983) Ascidies littorales de Guadeloupe. IV - Styelidae. *Bulletin du Muséum National d'Histoire Naturelle* 5(2): 423-456
- Monniot C (2002) Stolidobranch ascidians from the tropical western Indian Ocean. *Zoological Journal of the Linnean Society* 135: 65-120, doi:10.1046/j.1096-3642.2002.00017.x
- Monniot C, Monniot F (1983) Navigation ou courants? La colonisation des Açores et des Bermudes par les ascidies (tuniciers benthiques). *Comptes Rendus de la Société de Biogéographie* 59(1): 53-58



- Monniot C, Monniot F, Laboute P (1985) Ascidiés du port de Papeete (Polynésie française): relations avec le milieu naturel et apports intercontinentaux par la navigation. *Bulletin du Muséum National d'Histoire Naturelle* 7(3): 481-495
- Monniot F, Martoja R, Monniot C (1993) Accumulation d'étain dans les tissus d'ascidiés de ports méditerranéens (Corse, France). *Comptes Rendus de l'Académie des Sciences Paris* 361: 588-592
- Morris RH, Abbot DP, Haderlie EC (1980) Intertidal invertebrates of California. Stanford University Press, Stanford
- Moure JS, Björnberg TKS, Loureiro TS (1954) Protochordata ocorrentes na entrada da Baía de Paranaguá. *Dusenía* 5(5-6): 233-242
- Nakauchi M, Takeshita T (1983) Ascidian one-half embryos can develop into functional adult ascidians. *Journal of Experimental Zoology* 227(1): 155-158, doi:10.1002/jez.1402270120
- Naranjo AS, Carballo JL, García-Gomes JC (1996) Effects of environmental stress on ascidian population in Algeciras Bay (southern Spain). Possible marine bioindicators? *Marine Ecology Progress Series* 144: 119-131, doi:10.3354/meps144119
- Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York, USA, 512 pp.
- Nishikawa T (1980) Ascidiés from the coast of Kii Peninsula, Middle Japan, with description of two species. *Memoirs of the National Science Museum* 13: 97-111
- Nishikawa T (1991) The ascidiés of the Japan Sea. II. *Publications of the Seto Marine Biological Laboratory* 35(1-3): 25-170
- Oka A (1935) Report of the biological survey of Mutsu Bay 28. Ascidiés Simplicés. *Science Reports of Tohoku University* 10(4): 428-466
- Perera M, Ballesteros M, Turon X (1990) Estudio de los organismos epibiontes en un cultivo de bivalvos marinos del delta del Ebro. *Cahiers de Biologie Marine* 31: 385-399
- Pérès JM (1949) Contribution a l'étude des ascidiés de la côte occidentale d'Afrique. Bulletin de l'Institut Fondamental de l'Afrique Noire. Serie A. *Sciences Naturelles* 11(1-2): 159-207
- Pérès JM (1951) Nouvelle contribution à l'étude des Ascidiés de la côte occidentale d'Afrique. Bulletin de l'Institut Fondamental de l'Afrique Noire. Serie A. *Sciences Naturelles* 13(4): 1051-1071
- Pérès JM (1952) Les ascidiés de la roche littorale Corse. *Recueil des Travaux de la Station Marine d'Endoume* 6: 35-44
- Pérès JM (1954) Contribution a l'étude des ascidiés de Tunisie. *Bulletin de la Station Océanographique de Salammbó* 49: 3-19
- Pérès JM (1958a) Ascidiés récoltées sur les cotes méditerranéennes d'Israël. *Bulletin of the Research Council of Israel* 3-4(7B): 143-150
- Pérès JM (1958b) Ascidiés de la Baie de Haifa collectées par E. Gottlieb. *Bulletin of the Research Council of Israel* 3-4(7B): 151-164
- Pisut DP and Pawlik JR (2002) Anti-predatory chemical defenses of ascidiés: secondary metabolites or inorganic acids? *Journal of Experimental Marine Biology and Ecology* 270(2): 203-214, doi:10.1016/S0022-0981(02)00023-0
- Plough HH (1978) Sea Squirts of the Atlantic continental shelf from Maine to Texas. The Johns Hopkins University Press, Maryland, USA, 118 pp
- Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817-818, doi:10.1093/bioinformatics/14.9.817
- Raftos D, Hutchinson A (1997) Effects of common estuarine pollutants on the immune reaction of tunicates. *Biology Bulletin* 192: 62-72, doi:10.2307/1542576
- Ramos Espla AA, Buencuerpo V, Vazquez E, Lafarque F (1992) Some biogeographical remarks about the ascidian littoral fauna of the Strait of Gibraltar (Iberian sector). *Bulletin De L'Institut Océanographique Monaco* 9: 126-132
- Raymond M, Rousset F (1995) An exact test for population differentiation. *Evolution* 49: 1280-1283, doi:10.2307/2410454
- Reish DL (1963) Mass mortality of marine organisms attributed to the "red tide" in southern California. *California Fish Game* 49: 265-270
- Ritter WE, Forsyth RA (1917) Ascidiés of the littoral zone of southern California. *University of California Publications in Zoology* 16: 439-512
- Rocha RM, Costa LV (2005) Ascidiés (Urochordata: Ascidiacea) from Arraial do Cabo, Rio de Janeiro, Brazil. *Iheringia. Série Zoologia* 95(1): 57-64
- Rocha RM, Kremer LP (2005) Introduced ascidiés in Paranaguá Bay, Paraná, southern Brazil. *Revista Brasileira de Zoologia* 22(4): 1170-1184, doi:10.1590/S0101-81752005000400052
- Rodrigues AS (1962) Algumas ascidiés do litoral sul do Brasil. *Boletim da Faculdade de Filosofia Ciências e Letras da Universidade de São Paulo - Zoologia* 24: 193-216
- Roman J (2006) Diluting the founder effect: cryptic invasions expand a marine invader's range. *Proceedings of the Royal Society. Series B. Biological Sciences* 273: 2453-2459
- Roman J, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*, in Europe. *Molecular Ecology* 13: 2891-2898, doi:10.1111/j.1365-294X.2004.02255.x
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574, doi:10.1093/bioinformatics/btg180
- Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19: 2496-2497, doi:10.1093/bioinformatics/btg359
- Ruiz GM, Carlton JT, Grosholz ED, Hines AH (1997) Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist* 37: 621-632
- Saitou N, Nei M (1987) The Neighbor-joining Method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4(4): 406-425
- Sims LL (1984) Osmoregulatory capabilities of three macrosympatric stolidobranch ascidiés, *Styela clava* Herdman, *S. plicata* (Lesueur), and *S. montereyensis* (Dall). *Journal of Experimental Marine Biology and Ecology* 82(2-3): 117-129, doi:10.1016/0022-0981(84)90098-4
- Swofford DL (2000) PAUP\* Phylogenetic analysis using parsimony (\*and other methods). Sinauer, Sunderland
- Thiyagarajan V, Qian PY (2003) Effect of temperature, salinity and delayed attachment on development of the solitary ascidian *S. plicata* (Lesueur). *Journal of Experimental Marine Biology and Ecology* 290: 133-146, doi:10.1016/S0022-0981(03)00071-6
- Tokioka T (1951) Contributions to Japanese ascidian fauna. IV - Notes on some ascidiés collected in Osaka Bay.

- Publications of the Seto Marine Biological Laboratory* 1(4): 11-25
- Tokioka T (1953a) Ascidiens of Sagami Bay. Iwanami Shoten, Tokyo, 315 pp
- Tokioka T (1953b) Contributions to Japanese ascidian fauna. VI – Simple ascidiens of the Museum of Hukui. *Publications of the Seto Marine Biological Laboratory* 3(1): 27-32
- Tokioka T (1954) Contributions to Japanese ascidian fauna. X - Notes on some ascidiens collected in Osaka Bay. *Publications of the Seto Marine Biological Laboratory* 4(1): 75-105
- Tokioka T (1960) Contributions to Japanese Ascidian Fauna. XVII Ascidiens found in the benthonic samples dredged in the Ariake Sea 1957-58. *Publications of the Seto Marine Biological Laboratory* 8(1): 205-221
- Tokioka T (1963) Contributions to Japanese ascidian fauna. XX. The outline of Japanese ascidian fauna as compared with that of the Pacific coasts of North America. *Publications of the Seto Marine Biological Laboratory* 11(1): 131-155
- Tokioka T (1967) Pacific Tunicata of the United States National Museum. *United States National Museum Bulletin* 251: 1-247
- Tokioka T, Nishikawa T (1975) Contributions to Japanese ascidian fauna. XXVII - Some ascidiens from Okinawa, with notes on a small collection from Hong Kong. *Publications of the Seto Marine Biological Laboratory* 22(5): 323-341
- Traustedt MPA (1883) Vestindiske Ascidiæ simplices. Anden afdeling. Molgulidae og Cynthidae. *Videnskabelige Meddelelser fra Dansk Naturhistoriske Forening* 1882: 108-136
- Traustedt MPA, Weltner W (1894) Bericht über die von Herrn Dr. Sander gesammelten Tunicaten. *Archiv Naturges* 60: 10-14
- Tucker GH (1942) The histology of the gonads and development of the egg envelopes of an ascidian (*S. plicata* Lesueur). *Journal of Morphology* 70: 81-113, doi:10.1002/jmor.1050700106
- Turon X, Perera M (1988) Las ascidiens del delta del Ebro. Aspectos faunísticos y cuantitativos. *Publicaciones del Departamento de Zoología* 14: 81-90
- Turon X, Tarjuelo I, Duran S, Pascual M (2003) Characterizing invasion processes with genetic data: an Atlantic clade of *Clavelina lepadiformis* (Ascidiacea) introduced into Mediterranean harbours. *Hydrobiologia* 503: 29-35, doi:10.1023/B:HYDR.0000008481.10705.c2
- Tursi A (1976) Ascidiens de la Mer Jonienne des parages du nouveau port Italsider (Taranto) première note: campagne 1975. *Oebalia* 2(1): 153-163
- Tursi A, Liaci LC (1983) Settlement periods of ascidiens in the Mar Piccolo of Taranto in ten years 1972-1982. *Oebalia* 9: 65-80
- Tursi A, Gherardi M, Lepore E (1974) The breeding season of some ascidiens of “Mar Piccolo” (Taranto, Italy): preliminary data. *Memorie di Biologia Marina e di Oceanografia* 4(4-5-6): 421-434
- Van Name WG (1902) The ascidiens of the Bermuda Islands. *Transactions of the Connecticut Academy of Arts and Science* 11: 325-412
- Van Name WG (1912) Simple ascidiens of the coasts of New England and neighbouring British provinces. *Proceedings of the Boston Society of Natural History* 34: 439-619
- Van Name WG (1945) The North and South American Ascidiens. *Bulletin of the American Museum of Natural History* 84: 1-476
- Watterson GA, Guess HA (1977) Is the most frequent allele the oldest? *Theoretical Population Biology* 11(2): 141-160, doi:10.1016/0040-5809(77)90023-5
- Wyatt ASJ, Hewitt CL, Walker DI, Ward TJ (2005) Marine introductions in the Shark Bay World Heritage Property, Western Australia: a preliminary assessment. *Diversity and Distributions* 11: 1-33, doi:10.1111/j.1366-9516.2005.00109.x
- Yamaguchi M (1975) Growth and reproductive cycles of the marine fouling ascidiens *Ciona intestinalis*, *S. plicata*, *Botrylloides violaceus* and *Leptoclinum mitsukurii* at Abaratsubo-Moroiso Inlet (Central Japan). *Marine Biology* 29: 253-259, doi:10.1007/BF00391851
- Zanuy H (1990) Lista de ascidiens cubanas. Poeyana. Instituto de Ecología y Sistemática. *Academia Ciencias de Cuba* 388: 1-7

**Annex 1**Historical records of *Styela plicata* worldwide (Substrate type: A - artificial, N - natural, E – epibiosis).

Region	Country	Location (geographic coordinates)	Map ref.	Date	Subst rate	Source
North West Atlantic	Canada	?				Huntsman 1912
	USA	New York (40°42'N, 74°02'W)	1			De Kay 1843
	USA	Philadelphia (Harbor) (39°55'N, 75°08'W)	2		A	Lesueur 1823
	USA	North Carolina (Fort Macon, Beaufort) (35°05'N, 77°06'W)	3	1915		Tucker 1942; Van Name 1945; Abbott & Johnson 1972; Plough 1978
	USA	Georgia (Sapelo Island) (31°23'N, 81°17'W)	4	1968-74		Plough 1978
	USA	Florida (Cedar Key, Boca Ciega Bay) (29°15'N, 83°04'W)	5	1950		Abbott & Johnson 1972
	USA	Florida (Fort Pierce, Harbor Branch marina) (27°32'N, 80°20'W)	6	1987		Bingham & Walters 1989
	USA	Florida (Virginia Key) (25°44'N, 80°09'W)	7			Goodbody 1984
	Bahamas	Little San Salvador Island (24°34'N, 75°58'W)	8			Pisut & Pawlik 2002
	USA	Florida (Tortugas Islands) (24°37'N, 82°55'W)	9	1968-74		Plough 1978
	USA	Florida (Apalachee Bay) (30°06'N, 84°09'W)	10	1968-74		Plough 1978
	USA	Texas (Sea Ranch marina, Parrot Eyes marina, Port Isabel) (26°04'N, 97°10'W)	11	2004		Lambert et al. 2005
	Bermuda	St. David Island, Church Bay, Spanish Point, Harrington Sound (32°33'N, 65°12'W)	12			Van Name 1945; Monniot 1972; Monniot & Monniot 1983
Caribbean and Central America	Belize	Twin Cays (north Channel) (16°50'N, 88°06'W)	13	1981	E	Goodbody 2004
	Cuba	Gulf of Ana Maria (21°50'N, 80°00'W)	14			Van Name 1945; Zanuy 1990
	Jamaica	Port Royal (18°32'N, 77°22'W)	15			Goodbody 2003
	Puerto Rico	Puerto de Guánica (17°57'N, 66°54'W)	16			Van Name 1945
	St. Thomas, St Vincent	(18°21'N, 64°56'W)	17			Van Name 1945
	St. Croix	(45°30'N, 64°45'W)	18			Van Name 1945
	Guadalupe	Port Saint-François (16°25'N, 61°58'W)	19			Monniot 1983
	Isla Margarita	Puente de la Restinga (11°37'N, 64°17'W)	20			Goodbody 1984
South West Atlantic	Brazil	Bahia (Baía de Todos os Santos) (13°37'S, 38°48'W)	21	1998		Lotufo 2002
	Brazil	Rio de Janeiro (Cabo Frio e Arraial do Cabo) (23°28'S, 42°00'W)	22			Costa 1969; Lotufo 2002; Rocha & Costa 2005
	Brazil	Rio de Janeiro (Niteroi) (23°28'S, 43°10'W)	23			Millar 1958
	Brazil	Baía da Guanabara, Rio de Janeiro (23°37'S, 43°15'W)	23			Lotufo 2002; Traustedt 1883

## Annex 1 (continued)

Region	Country	Location (geographic coordinates)	Map ref.	Date	Subst rate	Source
South West Atlantic	Brazil	Rio de Janeiro (Ilha Grande) (23°07'S, 44°23'W)	24			Monniot 1969-1970
	Brazil	São Paulo (São Sebastião) (24°20'S, 45°41'W)	25		A	Rodrigues 1962
	Brazil	São Paulo (Santos) (24°33'S, 46°33'W)	25			Millar 1958
	Brazil	Paraná (Paranaguá Bay, Ilha do Mel) (26°00'S, 48°55'W)	26			Moure et al. 1954
	Brazil	Santa Catarina (Florianópolis) (28°12'S, 48°58'W)	27			Rodrigues 1962, present work
	Uruguay	Montevideo (34°55'S, 56°15'W)	28			Traustedt 1883
Mediterranean	Spain	Strait of Gibraltar (36°36'N, 5°55'W)	29	1989		Ramos Espla et al. 1992
	Spain	Algeciras Bay (36°13'N, 5°45'W)	29			Naranjo et al. 1996
	Spain	Ebro Delta (41°12'N, 1°13'W)	30	1986	A, E	Turon & Perera 1988
	Spain	Cabo de Tortosa, Catalonia (41°22'N, 0°56'E)	30		E	Perera et al. 1990
	France	Port de Banyuls, Port-Vendres (42°47'N, 3°12'E)	31		A, E	Fiala-Medioni 1974
	France	Nice (43°21'N, 7°21'E)	32	1964-65	A	Leung Tack Kit 1975
	France	Corsica (Ajaccio, Bonifacio) (41°38'N, 9°14'E)	33	1950	A	Pérès 1952; Monniot et al. 1993
	Tunisia	North lagoon (37°21'N, 10°18'E)	34	1953		Pérès 1954
	Italy	Naples (40°50'N, 14°15'E)	35			Traustedt 1883
	Italy	Mar Piccolo de Taranto (40°28'N, 17°15'E)	35	1972-82		Tursi et al. 1974; Tursi 1976; Tursi & Liaci 1983
	Italy	Venician Lagoon, Venice (45°44'N, 12°33'E)	36	1971-74		Brunetti 1979
	Italy	Adriatic sea (42°58'N, 16°28'E)	36	1994-96		Gabriele et al. 1997
	Egypt	Port Said, Suez Canal (31°15'N, 32°18'E)	37	1927		Ghobashy & Abdel Messeih 1991
	Israel	Mediterranean shore (31°50'N, 34°38'E)	38	1955	E	Pérès 1958a
Israel	Haifa Bay (32°48'N, 35°01'E)	38		N, E	Pérès 1958b	
Atlantic East	Mauritania	Port-Étienne, Cap Chacal, Baie de l'Étoile (21°01'N, 17°01'W)	39	1948		Pérès 1949
	Senegal	Dakar (Bel Air, Gorée, Tacoma) (14°40'N, 17°24'W)	40	1948		Monniot 1969; Pérès 1949
	Senegal	Dakar (Baia de Hann) (14°43'N, 17°25'W)	40	1950	A	Pérès 1951
Indian Ocean	South Africa	Durban, South Africa) (29°51'S, 31°02'E)	41	1951	A	Millar 1955
	Somalia	(9°41'N, 51°03'E)	42	1964	A	Millar 1988
	Suez Gulf	(30°28'N, 32°55'E)	43			Monniot, 2002
	Australia	Shark Bay (25°47'S, 113°41'E)	44			Kott 1972a; Wyatt et al. 2005

## Annex 1 (continued)

Region	Country	Location (geographic coordinates)	Map ref.	Date	Subst rate	Source
Indian Ocean	Australia	Western region (Cockburn Sound, Swan River, Canning River) (32°11'S, 115°43'E)	45			Kott 1985
	Australia	Western region (Bunbury) (33°18'S, 115°38'E)	45			Kott 1985
	Australia	Southern region (Coffin Bay) (34°58'S, 135°31'E)	46			Kott 1972b
	Australia	Southern region (Spencer Gulf, St. Vincent Gulf) (34°29'S, 138°15'E)	46			Kott 1985
North West Pacific	China	Hong Kong (Tolo Harbour, Mirs Bay) (22°25'N, 114°17'E)	47			Tokioka & Nishikawa 1975; Kott & Goodbody 1980; Thiagarajan & Qian 2003
	Japan	Mutsu Bay (41°08'N, 141°24'E)	48			Oka 1935
	Japan	Tsukumo Bay and Nanao Bay, Japan Sea (37°30'N, 137°23'E)	49			Nishikawa 1991
	Japan	Yokohama Harbor (Sagami Bay) (35°27'N, 139°58'E)	50		N	Tokioka 1953a
	Japan	Bay of Tokyo, Obama Bay (35°35'N, 139°51'E)	50			Traustedt & Weltner 1894; Tokioka 1953b
	Japan	Sakushima Island (34°43'N, 137°02'E)	50	2006		Present study
	Japan	Osaka Bay (34°55'N, 135°28'E)	51	1950-52		Tokioka 1951, 1954
	Japan	Kii Peninsula (34°31'N, 135°01'E)	51	1963		Nishikawa 1980
	Japan	Nagasaki (32°45'N, 129°47'E)	52			Tokioka 1963
Japan	Ariake sea, Kyūsyū Island (33°00'N, 131°00'E)	53	1957-58	E	Tokioka 1960	
South West Pacific	Australia	Queensland (Moreton Bay, Harvey Bay, Ross Creek, Calliope River) (27°33'S, 153°25'E)	54	1970-71		Kott 1972a; Kott 1985
	Australia	Sydney Harbor (33°51'S, 151°12'E)	55	1877		Heller 1878; Raftos & Hutchinson 1997
	Australia	New South Wales (Garden Island, Botany Bay, Port Kembla) (34°07'S, 151°17'E)	55	1948-49		Kott 1952; Kott 1973; Kott 1985
	Australia	Victoria (Port Phillip Bay) (38°03'S, 144°51'E)	56	1958		Millar 1966; Kott 1985
	New Zealand	Gulf of Hauraki (36°41'S, 175°05'E)	57	1948		Brewin 1948; Brewin 1958, Millar 1982
North East Pacific	USA	California (Santa Barbara Harbor, King Harbor, Alamitos Harbor, San Pedro, Mission Bay) (34°40'N, 120°08'W)	58		A, N	Reish 1963; Fay & Johnson 1971
	USA	California (Long Beach, Newport Bay) (34°18'N, 118°25'W)	58	1950		Abbott & Johnson 1972
	USA	California (San Diego) (33°23'N, 117°17'W)	58	1915	E	Ritter & Forsyth 1917