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Cryptic speciation in a high gene flow scenario in the oviparous marine sponge *Chondrosia reniformis*

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Abstract Sponge systematics has been traditionally based on the study of the skeleton (spicules and spongin fibres). However, sponges of the genus *Chondrosia* are devoid of those skeletal features, making it difficult to distinguish between different species in the genus. *Chondrosia reniformis* Nardo, 1847, the type species of the genus, was described from the Mediterranean Sea. The lack of distinguishing morphological features may have been responsible for the widespread assignment of specimens of the genus to this species; as a result *C. reniformis* is considered to be a cosmopolitan species. In this work, populations of *C. reniformis* from the western Mediterranean (France) and the West Atlantic (Bermuda and Brazil) were analysed using allozyme electrophoresis for 13 enzyme loci. Levels of mean heterozygosity were high (Bermuda and Brazil $H = 0.27$ and W Mediterranean $H = 0.12$), as is often observed in sponge species. Gene identities observed between West Atlantic and Mediterranean populations were low ($I = 0.40$–0.52, typical values for congeneric species), including the presence of four diagnostic loci. This level of divergence clearly shows that they are not conspecific. Hence, a worldwide or cosmopolitan distribution of *C. reniformis* would seem improbable. However, the West Atlantic samples (Bermuda and Brazil) were genetically similar (gene identity, $I = 0.88$–0.95) over a distance of 8,000 km. This is the first report of genetic homogeneity in a sponge species over such a large geographical distance.

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Introduction

Many benthic invertebrate species have been considered to have a worldwide distribution. This was supported by a belief that marine invertebrate larvae, often associated with planktonic dispersal, were able to disperse over long distances (e.g. Strathmann 1985; Scheltema1986). The capacity for long-range dispersal of larvae was usually inferred from a simple calculation, whereby the laboratory-measured duration of larval life was multiplied by average speeds of surface currents (Jablonski 1986). However, larval behaviour (Maldonado and Young 1996), predation (Olson and McPherson 1987) and macro- and micro-hydrodynamic aspects of dispersal (Cowen et al. 2000) were often ignored in calculations, and recent studies suggest that larvae often disperse far less than their potential (Jackson 1986; Knowlton and Keller 1986; Ayre and Hughes 2000). Not surprisingly in these cases, genetic studies of supposedly cosmopolitan taxa have often revealed the presence of complexes of sibling species (for reviews see, e.g. Knowlton 1993, 2000; Thorpe and Solé-Cava 1994). Nevertheless, other studies have revealed surprisingly little geographic differentiation across large distances in taxa with limited dispersal potential and which settle shortly after release (Solé-Cava et al. 1994; Grant and da Silva-Tatley 1997). No such examples are known for marine sponges, however, a group whose larvae probably disperse poorly (Borojevic 1970; Sarà and Vacelet 1973; Uriz 1982; for an exception see Vacelet 1999); all previous genetic analyses of sponge populations have revealed differentiation across distances ranging from 1 to 2,700 km. Above that distance, the degree of genetic differentiation typically suggests that supposedly con-

specific forms merit recognition at the species level (e.g. Solé-Cava et al. 1991; Boury-Esnault et al. 1992, 1999; Klautau et al. 1999). The fact that these forms went unrecognised previously has been attributed to over-conservative taxonomy, related primarily to the small number of morphological characters available for classification, and to an overestimation of the dispersal ability of sponge larvae (Klautau et al. 1999; Solé-Cava and Boury-Esnault 1999).

Chondrosia reniformis Nardo, 1847 is the type species of a genus that is characterised by the absence of skeleton, the main morphological character used in the systematics of sponges. This species was described from the Mediterranean Sea, and, since then, it has been allegedly identified worldwide, including the Indian, Pacific, and East and West Atlantic Oceans (for a review see Wiedenmayer 1977). *C. reniformis* is a common species that lives in littoral zones (0–50 m), usually on shaded walls (Wilkinson and Vacelet 1979). As a result of its ubiquity and ecological importance, there are several studies of the biology or ecology of *C. reniformis* (e.g. Wilkinson and Vacelet 1979; Bavestrello et al. 1995, 1998; Sarà et al. 1998). Levels of gene variation have been already estimated within a Mediterranean population of *C. reniformis* (Solé-Cava and Thorpe 1991), and the phylogenetic position of the genus within the demosponges has been inferred by DNA sequencing (Chombard 1998; Vacelet et al. 2000). However, to date, no information is available on the levels of genetic differentiation between geographically distant populations of any species of *Chondrosia*.

C. reniformis, in the Mediterranean, is gonochoric and oviparous, but it is also known to reproduce asexually (Scalera-Liaci and Sciscioli 1975; Bavestrello et al. 1995, 1998). The dispersal capability of the lecithotrophic larva of *C. reniformis* is probably low, as has been suggested for many other sponge larvae (Borojevic 1970; Sarà and Vacelet 1973; Uriz 1982). Gamete dispersal is also likely to be very limited, since, after release, oocytes remain close to the parent, and spermatozoa are thought to remain in the water column for a maximum of only a fewhours (Lévi and Lévi 1976). Recently, high levels of population structuring ($F_{ST} = 0.21$) along about 2,700 km of Brazilian coast were found in the confamilial genus *Chondrilla* (Klautau et al. 1999). Furthermore, four cryptic species were found within the supposedly cosmopolitan "*Chondrilla nucula*" in the Brazilian/Caribbean area (Klautau et al. 1999). These results, once again, point to the low realised dispersal of sponge larvae, and to the need to re-evaluate genetically the validity of species with supposedly large geographic distributions (Thorpe and Solé-Cava 1994).

Allozymes have been used successfully as a complementary tool in the identification of cryptic species in the phylum Porifera (reviewed in Solé-Cava and Boury-Esnault 1999). The general trend observed in these studies is that speciation in sponges may be accompanied by a much smaller level of morphological divergence than that traditionally considered by systematists to indicate differentiation at the species level (Klautau et al. 1994, 1999; Boury-Esnault et al. 1999; Solé-Cava and Boury-Esnault 1999).

In this paper, we used allozyme electrophoresis to demonstrate that *C. reniformis* from the western Atlantic (Bermuda and Brazil) and from the western Mediterranean (France) are not conspecific. Contrastingly, populations of western Atlantic "*C. reniformis*", separated by more than 8,600 km, were genetically remarkably similar. This is the first record of high genetic similarity in a sponge species over a large geographic distance.

Materials and methods

Sample collection

Between June 1996 and September 1997, 159 individuals of *Chondrosia reniformis* (Demospongiae: Chondrillidae) were collected, by snorkelling or SCUBA diving, from nine localities (Fig. 1) in the western Atlantic: Bermuda (32°18′N; 64°46′W); Brazil (Recife 08°07′S; 34°52′W; Búzios 22°44′S; 41°53′W; Praia do Forno 22°58′S; 42°01′W; Angra dos Reis 23°01′S; 44°18′W); and the Mediterranean, on the French coast (Provence: La Vesse 43°21′N; 05°15′E; Endoume 43°16′N; 05°20′E; Callelongue 43°10′N; 05°23′E; La Ciotat 43°10′N; 05°35′E). Care was taken to avoid collecting individuals closer than 2 m apart, thus minimising the probability of collecting clone-mates. Furthermore, the genotypes of all individuals from each site were compared, treating any individuals with the same compound genotype as ramets of a single genet (sensu Harper 1977). This resulted in the exclusion of two individuals, from the populations of Recife and Forno on the Brazilian coast.

The specimens were transported alive or on ice to the laboratory and stored in liquid nitrogen until required for electrophoresis, or in 70% ethanol for taxonomic identification.

Geographic distances between sampling sites (measured as lowest spherical distances by sea), and their co-ordinates, were calculated using the Microsoft programme "Encarta 99 Atlas".

Electrophoresis

Horizontal 12.5% starch gel electrophoresis was carried out as previously described for sponges (e.g. Solé-Cava and Thorpe 1986; Klautau et al. 1999). The buffer systems used were: 0.10 M Tris, 0.01 M EDTA, 0.10 M maleate, pH 7.4 (TEM; Brewer 1970); and 0.06 M NaOH, 0.30 M borate, pH 8.1 (gel), 0.076 M Tris, 0.005 M citrate, pH 8.7 (electrode) (POULIK; Poulik 1957). Nine, out of 30, enzyme systems investigated produced consistent and reproducible results in all populations: catalase (*CAT*; EC 1.11.1.6); diaphorase (*DIA*; EC 1.8.1.4); esterases (*EST*; EC 3.1.1.1); hexokinase (*HK*; EC 2.7.1.1); malate dehydrogenase (*MDH*; EC 1.1.1.37); mannosephosphate isomerase (*MPI*; EC 5.3.1.8); peptidases (*PEP*; EC 3.4.1.1); phosphoglucose isomerase (*PGI*; EC 5.3.1.9); and phosphoglucomutase (*PGM*; EC 5.4.2.2). The staining of the gels followed standard procedures (Manchenko 1994). Non-specific bands, as previously reported for other sponge species (e.g. Stoddart 1989; Boury-Esnault et al. 1992; Klautau et al. 1999), were observed in all samples. These bands were not used for the genetic analyses, since their origin remains unclear.

Genotype frequency data were used to estimate gene frequencies, levels of gene variation (heterozygosity, *H*), fits to Hardy–Weinberg equilibrium (F_{IS}; Wright 1978), inbreeding indices (F_{ST}; Wright 1978), and pairwise unbiased gene identities (*I*) and distances (*D*) (Nei 1978), using the BIOSYS-1 programme, version 1.7 (Swofford and Selander 1981). The significance of F_{IS} (H_o:$F_{IS} = 0$) and F_{ST} (H_o:$F_{ST} = 0$) were estimated using a χ^2 test (Waples 1987). Effective number of migrants ($N_e m$) was estimated as $N_e m \approx [(1/F_{ST}) - 1]/4$ (Wright 1978). Although this estimate relies

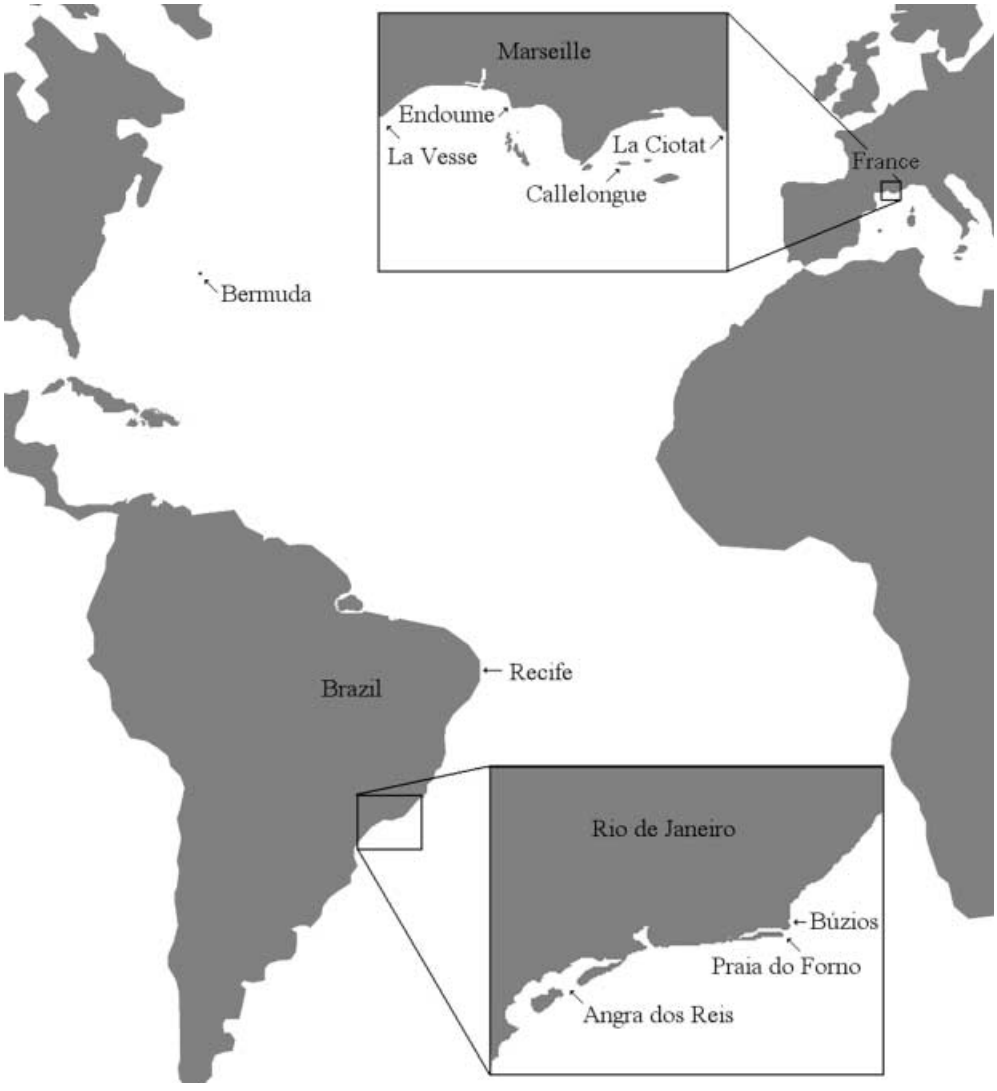

Fig. 1 *Chondrosia reniformis*. Sampling sites

upon a high number of assumptions, like mutation-drift equilibrium and neutrality (Bossart and Prowell 1998; Whitlock and McCauley 1999), it can still be useful, if only as a rough indicator of present or recent levels of gene flow between populations (Bohonak et al. 1998; Bohonak 1999; Ayre and Hughes 2000).

As there are large stochastic errors associated with the estimation of gene identities over small numbers of loci (Nei 1987), we used the UPGMA algorithm (Sneath and Sokal 1973) to construct a dendrogram of relationships between populations, since this method has been shown to give better estimates of tree topology when the variance is large (Nei et al. 1983).

Finally, to investigate whether geographic distances were directly correlated to genetic distances (Nei 1978) between pairs of *Chondrosia* populations, we used a Mantel test, with 1,000 replicates (Sokal and Rohlf 1995).

Results

Gene frequencies and heterozygosity estimates of *Chondrosia reniformis* populations are shown in Table 1. Four of the 13 gene loci observed (*CAT-1*, *EST-1*, *MDH* and *PGM*; Table 1) were fixed for different alleles in the populations from the West Atlantic (Bermuda and Brazil) and the western Mediterranean. Consequently, levels of gene identity between those populations were very low ($I = 0.40$–0.52; Table 2; Fig. 2). On the other hand, gene identities between Mediterranean populations, and between populations from Brazil and Bermuda were high ($I = 0.96$–0.99 and $I = 0.88$–0.95, respectively; Table 2; Fig. 2). Despite their high genetic similarity, *C. reniformis* populations were found to be genetically structured both in the western Atlantic ($F_{ST} = 0.16$; $P < 0.0001$) and the Mediterranean ($F_{ST} = 0.13$; $P < 0.0001$).

Genotype frequencies did not depart from Hardy–Weinberg expectations at any of the loci studied ($F_{IS} = 0.03$–0.10, $P > 0.70$; Fisher's exact-test, $P > 0.05$ – using a Bonferroni transformation for multiple tests; Lessios 1992).

No significant correlation was observed between geographic distance and genetic differentiation between Atlantic populations of *C. reniformis* (Mantel test, $P > 0.60$; Sokal and Rohlf 1995).

Table 1 *Chondrosia reniformis*. Gene frequencies of 13 allozyme loci (*N* number of individuals analysed; H_o, H_e: observed and Hardy–Weinberg expected heterozygosities, respectively)

Locus	Bermuda	Brazil				France			
		Recife	Búzios	Forno	Angra	La Vesse	Endoume	Callelongue	La Ciotat
CAT-1									
(*N*)	(16)	(7)	(6)	(19)	(13)	(5)	(25)	(14)	(9)
A	–	–	–	–	–	1.00	1.00	1.00	1.00
B	1.00	1.00	1.00	1.00	1.00	–	–	–	–
CAT-2									
(*N*)	(21)	(7)	(10)	(25)	(20)	(5)	(26)	(12)	(7)
A	–	0.07	–	–	0.10	0.30	0.32	0.38	0.21
B	0.48	0.72	0.90	0.52	0.60	–	0.33	0.12	0.36
C	0.52	0.21	0.10	0.46	0.30	0.70	0.29	0.38	0.43
D	–	–	–	0.02	–	–	0.06	0.12	–
DIA									
(*N*)	(18)	(7)	(10)	(24)	(18)	(5)	(26)	(14)	(8)
A	1.00	1.00	0.85	0.94	1.00	1.00	1.00	1.00	0.75
B	–	–	0.15	0.06	–	–	–	–	0.25
EST-1									
(*N*)	(13)	(3)	(9)	(16)	(18)	(2)	(20)	(9)	(6)
A	–	–	–	–	–	1.00	1.00	1.00	1.00
B	1.00	1.00	1.00	1.00	1.00	–	–	–	–
EST-2									
(*N*)	(13)	(7)	(9)	(22)	(18)	(2)	(27)	(14)	(8)
A	0.50	1.00	0.50	0.84	0.44	1.00	1.00	1.00	1.00
B	0.50	–	0.50	0.16	0.56	–	–	–	–
EST-3									
(*N*)	(13)	(7)	(9)	(22)	(18)	(5)	(28)	(14)	(8)
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
EST-4									
(*N*)	(13)	(7)	(9)	(22)	(18)	(5)	(28)	(14)	(8)
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
HK									
(*N*)	(23)	(2)	(10)	(29)	(19)	(2)	(21)	(9)	(8)
A	0.11	–	–	0.16	–	–	0.07	–	0.12
B	0.33	–	–	–	0.26	1.00	0.93	1.00	0.88
C	0.41	0.75	0.35	0.36	0.50	–	–	–	–
D	0.13	0.25	0.45	0.48	0.24	–	–	–	–
E	0.02	–	0.20	–	–	–	–	–	–
MDH									
(*N*)	(17)	(6)	(10)	(35)	(11)	(5)	(28)	(14)	(9)
A	0.12	–	0.35	–	–	–	–	–	–
B	0.29	0.67	0.35	0.63	1.00	–	–	–	–
C	0.47	0.33	0.30	0.18	–	–	–	–	–
D	0.12	–	–	0.19	–	–	–	–	–
E	–	–	–	–	–	1.00	0.96	1.00	1.00
F	–	–	–	–	–	–	0.04	–	–
MPI									
(*N*)	(21)	(3)	(10)	(39)	(19)	(2)	(20)	(9)	(9)
A	0.38	–	0.45	0.13	0.18	–	–	–	–
B	0.62	0.17	0.30	0.79	0.37	–	–	–	–
C	–	0.83	0.25	0.08	0.45	–	0.28	–	0.33
D	–	–	–	–	–	1.00	0.72	1.00	0.67
PEP									
(*N*)	(21)	(7)	(10)	(31)	(20)	(2)	(21)	(9)	(8)
A	0.05	–	0.25	0.08	–	–	–	–	–
B	0.50	1.00	0.60	0.79	0.98	1.00	1.00	1.00	1.00
C	0.45	–	0.15	0.13	0.02	–	–	–	–
PGI									
(*N*)	(22)	(7)	(10)	(39)	(19)	(4)	(28)	(13)	(9)
A	0.28	0.21	0.15	–	–	–	–	–	–
B	0.18	0.43	0.30	0.46	–	–	–	0.04	–
C	0.27	0.36	0.55	0.21	0.47	0.25	0.23	0.61	–
D	0.27	–	–	0.18	0.35	0.75	0.57	0.35	0.56
E	–	–	–	0.15	0.18	–	0.20	–	0.44

Table 1 (Continued)

Locus	Bermuda	Brazil				France			
		Recife	Búzios	Forno	Angra	La Vesse	Endoume	Callelongue	La Ciotat
PGM									
(*N*)	(12)	(7)	(10)	(30)	(11)	(5)	(28)	(14)	(8)
A	–	0.07	–	0.03	–	–	–	–	–
B	1.00	0.93	1.00	0.97	1.00	–	–	–	–
C	–	–	–	–	–	1.00	1.00	1.00	1.00
H_o	0.31	0.18	0.31	0.22	0.15	0.08	0.14	0.09	0.13
H_e	0.32	0.20	0.33	0.27	0.23	0.07	0.15	0.09	0.18

Discussion

The low genetic identity ($I = 0.40$–0.52) and the presence of four diagnostic loci between populations of *Chondrosia reniformis* from the western Atlantic (Bermuda and Brazil) and from the western Mediterranean clearly demonstrate that these are reproductively isolated and are evolving independently and, therefore, must be considered as separate biological species. Since the type locality of *C. reniformis* is the Mediterranean (Adriatic Sea), that species name must be associated with the Mediterranean samples, and, pending a formal description of the new species, we will refer to the western Atlantic specimens of "*C. reniformis*" as *Chondrosia* sp.

It could be argued that *Chondrosia* sp. might in fact be conspecific with one of the two *Chondrosia* species already cited for the tropical Atlantic region: *C. plebeja* Schmidt, 1868 or *C. collectrix* Schmidt, 1870. However, *Chondrosia* sp. is morphologically distinct from those two species (Table 3) and, indeed, from any other species of the genus *Chondrosia*. Therefore, the specimens from Bermuda and Brazil analysed here may be presumed to belong to a new species of *Chondrosia*, with a wide distribution in the western Atlantic. *Chondrosia* sp. and *C. reniformis* are morphologically virtually identical. Detailed descriptions of both species will be presented elsewhere.

The West Atlantic Oceanand the Mediterranean Sea belong to different biogeographic provinces, separated by large geographic distances and complex oceanic circulation patterns (Cuesta and Schubart 1998). The finding that amphi-Atlantic *C. reniformis* populations comprise at least two species is, therefore, not surprising, and is well within what seems to be the normal pattern for genetic comparisons of "cosmopolitan" sponge species (for review see Solé-Cava and Boury-Esnault 1999). In contrast, populations of *Chondrosia* sp. from Bermuda and Brazil were found to be genetically similar over a distance up to 8,600 km ($I = 0.88$–0.95).

The levels of gene identity observed between the Brazilian and Bermudan *Chondrosia* sp. were within the normal range observed in comparisons between conspecific populations (Thorpe and Solé-Cava 1994; Solé-Cava and Boury-Esnault 1999). F_{ST} values, although significantly different from zero, indicated a migration rate between sub-populations greater than one individual per generation ($N_e m = 1.27$). A high genetic similarity is usually interpreted as the result of present day gene flow between localities, although this may be surprising for a marine sponge with supposedly low dispersal capabilities. There are, however, alternative explanations, based on violations of the assumptions of the gene flow/population structuring models (absence of selection, and drift/mutation/inbreeding equilibrium). For example, populations are unlikely to be in equilibrium if they have originated recently, as in the case of anthropogenic-mediated bioinvasions (Holland 2000), or when they have undergone drastic and recurrent changes in size along their history(Lessios et al. 1994; Grant and da Silva-Tatley 1997). In such cases, levels of gene flow between populations will be greatly overestimated, because those populations simply have not had enough time to diverge (for a discussion of the problems of non-equilibrium processes see Davies et al. 1999). Anthropogenic transport has now been reported for

Table 2 *Chondrosia reniformis*. Unbiased genetic distances (*below diagonal*) and gene identities (*above diagonal*) (Nei 1978) between populations

Population	1	2	3	4	5	6	7	8	9
1. Bermuda	*****	0.882	0.947	0.945	0.918	0.482	0.491	0.478	0.475
2. Recife	0.126	*****	0.930	0.937	0.940	0.469	0.525	0.492	0.512
3. Búzios	0.054	0.073	*****	0.933	0.932	0.404	0.454	0.438	0.436
4. Forno	0.057	0.065	0.070	*****	0.934	0.475	0.497	0.476	0.492
5. Angra	0.086	0.062	0.071	0.068	*****	0.483	0.518	0.491	0.503
6. La Vesse	0.730	0.756	0.907	0.745	0.729	*****	0.985	0.988	0.974
7. Endoume	0.712	0.644	0.791	0.699	0.657	0.015	*****	0.984	0.995
8. Callelongue	0.739	0.710	0.825	0.743	0.711	0.012	0.016	*****	0.961
9. La Ciotat	0.745	0.669	0.829	0.708	0.688	0.027	0.005	0.040	*****

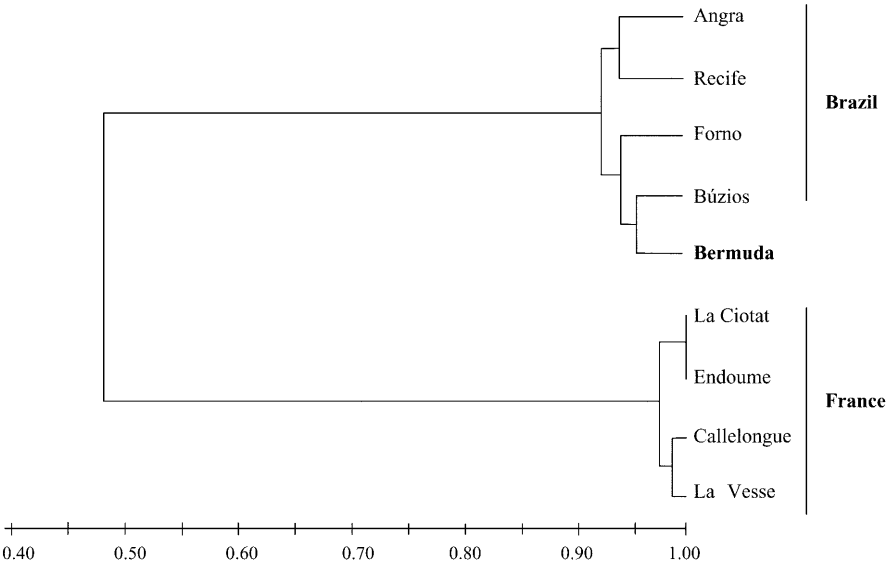

Fig. 2 *Chondrosia reniformis*. UPGMA dendrogram based on gene identities (Nei 1978) between populations

many marine invertebrate species and has been used as the main explanation for the unexpected increases in distribution (Holland 2000). However, no sponge larvae have been reported in the ballast water of cargo ships (e.g. Carlton and Geller 1993), and rafting seems to be very rare for sponges(for an exception see Maldonado and Uriz 1999); also, *C. reniformis* has never been considered a fouling species (Sarà and Orsi 1974). Moreover, invading populations usually have reduced levels of heterozygosity due to founder effects (Davies et al. 1999), whereas the populations of *Chondrosia* sp. studied here had higher levels of gene variation than those of *C. reniformis* from the Mediterranean Sea (Table 1).

An alternative explanation for the high genetic similarity observed could be a non-neutrality of the molecular markers used. For example, the low levels of gene differentiation found between populations of *Crassostrea virginica* from the Atlantic coastof the USA and the Gulf of Mexico were interpreted as resulting from high dispersal of oyster larvae (Buroker 1983). However, subsequent genetic studies, using mitochondrial DNA and nRFLPs, revealed high genetic differentiation between these *C. virginica* populations (Reeb and Avise 1990; Karl and Avise 1992, but see McDonald et al. 1996). The discrepancy between those studies was explained as the result of balancing selection acting on the allozyme loci (Reeb and Avise 1990, but see Hare and Avise 1998). However, in the case of *Chondrosia* sp., it is very difficult to envisage a homogeneous balancing selection scenario at so many gene loci and over such a wide geographical range.

It appears, thus, that in the absence of evidence for balancing selection or for anthropogenic-mediated dispersal, the high genetic similarity observed between *Chondrosia* populations along the Atlantic coast of North and South America should be interpreted as the result of the recent or current maintenance of some gene flow ($N_e m \approx 1$) between populations from those localities. This estimated effective number of migrants is usually considered to be sufficient to preclude significant long-term differentiation between populations (Wright 1978). Nevertheless, the populations of *Chondrosia* sp. studied do not seem to constitute a single panmictic population, displaying significant F_{ST} values. Similar levels of population structure ($F_{ST} = 0.05$–0.36) were found for dictyoceratid sponge

Table 3 *Chondrosia* spp. Diagnostic characters between species cited in the amphi-Atlantic region. "Inclusions" are calcareous fragments (sand grains), foreign spicules, corals, etc. [References: *1* Topsent (1896); *2* Topsent (1929); *3* Laubenfels (1936); *4* Mothes de Moraes and Bastian (1993); *5* Topsent (1918); *6* Wiedenmayer (1977); *7* present work]

Species	Cortex	Inclusions	Type locality	Cited locality	Ref.
C. reniformis Nardo, 1847	Smooth, 1–3 mm thick	Rare	Adriatic	Mediterranean	1, 2, 7
C. collectrix Schmidt, 1870	Irregular, 0.25 mm thick	Sometimes present in cortex or choanosome	Caribbean	West Atlantic: Bermuda, Caribbean and Brazil (NE)	3, 4
C. plebeja Schmidt, 1868	Polygonal folds, 1–10 mm thick	Present in abundance in cortex or choanosome	Algeria	Mediterranean, tropical East Atlantic and Caribbean	5, 6
Chondrosia sp. present paper	Smooth, 1–2 mm thick	Absent	–	West Atlantic: Brazil and Bermuda	7

species in Australia, but over a smaller geographic scale (700 km; Benzie et al. 1994).

Although the high genetic similarity over a large area is unexpected given the supposedly low dispersal capability of the larvae of *C. reniformis* (Lévi and Lévi 1976), this is not an isolated case among marine invertebrates. Genetic mixing of anti-tropical planktonic foraminiferan populations of three subpolar species was recently revealed by rDNA analysis. In those species at least one identical genotype was found in both Arctic and Antarctic regions, and bipolar populations of each species clustered tightly together in a phylogenetic analysis (Darling et al. 2000). Also, sea anemone populations of *Actinia bermudensis*, from Bermuda and along 2,000 km of Brazilian coast, have a relatively high genetic identity ($I = 0.82$), and were, hence, considered to be conspecific (Vianna 1999). That result was also unexpected considering the low dispersal capabilities of *A. bermudensis* (Russo et al. 1994; Monteiro et al. 1998). A similar result has been found, although on a smaller scale, in an East Atlantic sea anemone species (*Urticina eques*) with short-lived crawling larvae, for which populations from the North and Irish Seas (1,200 km apart) were found to be genetically very similar ($I > 0.95$; Solé-Cava et al. 1994).

The Brazilian and the Caribbean areas have been considered by many authors as parts of the same biogeographic province, due to their faunal similarities (Ekman 1953). It has been suggested that some genetic interchange has occurred between the two areas since the Pliocene (Vermeij and Rosenberg 1993). Such interchange could be facilitated, for example, by the almost continuous sub-littoral reef belt that exists along the north-eastern coast of South America up to the Caribbean (Kempf 1970, 1974). Therefore, reefs could form a bridge that would facilitate gene transfer between North- and Southwest Atlantic populations of *Chondrosia* sp. (although no correlation was found in our data between geographic and genetic distances) and other tropical benthic invertebrates. On the other hand, some supposed pan-American tropical Atlantic species have turned out to be, on closer scrutiny, complexes of different biological species (e.g. Sarver et al. 1998; Klautau et al. 1999). Other molecular studies are clearly needed to evaluate the degree of endemism of the benthic faunas of the tropical area of the North- and Southwest Atlantic coasts.

Since the West Atlantic sponge populations, originally attributed to *C. reniformis*, are genetically different from Mediterranean *C. reniformis*, it is unlikely that other citations of this species, in more distant places like Indonesia, the Red Sea and the Galapagos are correct. It is possible that *C. reniformis*, as with *Chondrilla nucula*, will become recognised as a species complex. Nonetheless, the high genetic similarity observed between *Chondrosia* sp. populations from widely separated areas in the West Atlantic shows that at least some sponge species may have a wide geographical distribution.

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