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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.

**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; Scheltema 1986). 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 few hours (Lévi and Lévi 1976). Recently, high levels of population structuring (*F*<sub>ST</sub> = 0.21) along about 2,700 km of Brazilian coast were found in the congeneric 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°32‘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 genotype (see 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% ethanolic 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 1991; 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; Poulík 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); phosphoglucone 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*<sub>IS</sub>; Wright 1978), inbreeding indices (*F*<sub>IS</sub>; Wright 1978), and pairwise unbiased gene identities (*D*) and distances (*D*) (Nei 1978), using the BIOSYS-1 programme, version 1.7 (Swofford and Selander 1981). The significance of *F*<sub>IS</sub> (*H<sub>0</sub>*<sub>IS</sub> = 0) and *F*<sub>ST</sub> (*H<sub>0</sub>*<sub>ST</sub> = 0) were estimated using a χ<sup>2</sup> test (Waples 1987).

Effective number of migrants (*N<sub>em</sub>*) was estimated as *N<sub>em</sub>* ≈ (1/*F*<sub>ST</sub>) – 1/4 (Wright 1978). Although this estimate relies